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ANNUAL RESEARCH PROGRESS REPORT.(U)
SEP 79

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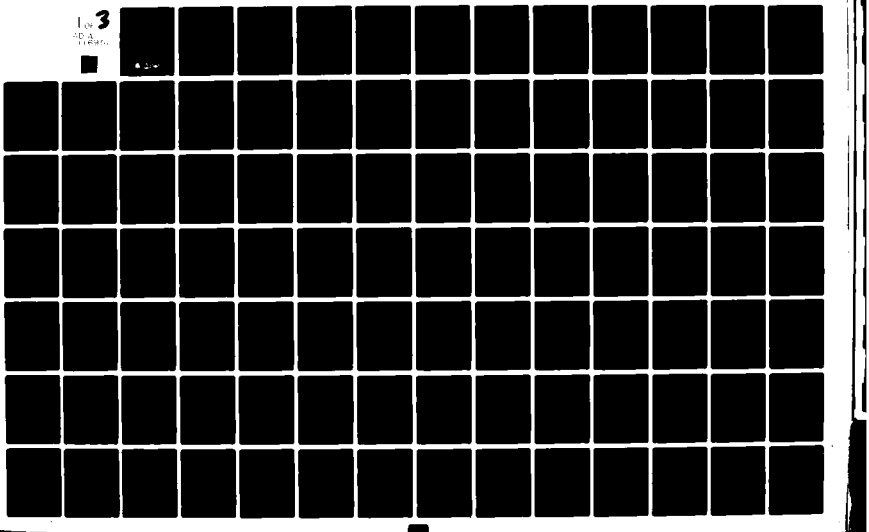

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ANNUAL RESEARCH PROGRESS REPORT

RCS-MEDDH-288(R1)

30 SEPTEMBER 1979

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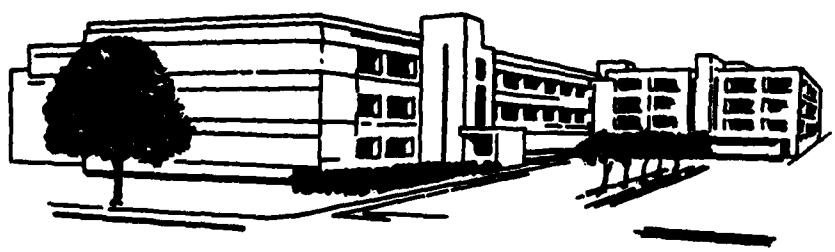

LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

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Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
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9. PERFORMING ORGANIZATION NAME AND ADDRESS Letterman Army Institute of Research Presidio of San Francisco, California 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
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18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) During Fiscal Year 1979 progress was attained at the Letterman Army Institute of Research in the following research areas: basic and applied studies on blood, blood products storage and blood substitutes; the effects of hemorrhagic shock on the heart; studies on the repairs of musculoskeletal structures; the determination of exposure thresholds of coherent radiation producing damage to the eye and skin; work performance of man and military dogs; the evaluation of insect repellent; serodiagnosis of leishmaniasis; mammalian toxicology and research computer		

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

science. The progress made in this fiscal year is described in the reports of the work units presented.

FOREWORD

The research conducted at the Letterman Army Institute of Research, Presidio of San Francisco, California, was accomplished in Fiscal Year 1979 under the following Department of the Army projects:

3A161101A91C - In House Laboratory Independent Research

3M161102BS01 - Basic Research on Military Injury and Diseases

3M161102BS02 - Basic Mechanisms of Recovery from Injury

3M162772A810 - Military Skin Disease

3M162772A812 - Military Research Animal Resources

3S162772A814 - Military Trauma and Resuscitation

Projects are subdivided into work units and studies, as appropriate, to accomplish project objectives.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

TABLE OF CONTENTS

Table of Contents (Cont)

Table of Contents (Cont)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6104	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ENTRY INSTRN	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 22	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	074 APC 505N
b. CONTRIBUTING	61101A	3A161101A91C	00	042
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Long Term Cryopreservation of Platelets for Immediate Field Use

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 012900 Physiology; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
76 01	cont.	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	4.2	121
FISCAL YEAR CURRENT 80	5.0	129

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Peck, Carl C., LTC, MC

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Provide EACH with Security Classification Code) (U) platelet storage; (U) cryopreservation; (U) blood storage; (U) massive transfusion; (U) platelet transfusion; (U) traumatic hemorrhage

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The need for effective hemostasis in severe combat injuries requires the availability of timely, effective component hemotherapy with coagulation factors and platelets. The former can be provided with relative ease but the latter, being perishable (72 hour storage limit) are logistically difficult to provide to rear line (even CONUS) medical facilities, and even more so to forward resuscitation units. This study is designed to develop and test storage systems whereby effective clinical doses of platelets can be stored, frozen for long periods of time, when easily thawed ready for immediate or delayed transfusion.

24. (U) Previous attempts under this work unit have been the development of new combinations of cryoprotective agents but have been frustrated by the lack of in vitro and animal in vivo tests to evaluate systems. The objectives are modified accordingly: 1) Evaluate full size clinical freezing protocols as to military objectives, feasibility, and possible modifications; 2) Control the multiple variable protocols as to total dose frozen to obtain therapeutic levels in one bag with the development of post-thaw suspension media to maintain viability up to 25 hours; 3) Develop human in vivo data on promising, safe protocols; 4) Correlation of new in vitro tests as predictors of successful storage.

25. (U) 78 09 - 79 10. a) Establish actual freezing protocol using 4% glycerol-5% glucose as the cryoprotectant with no-wash post-thaw properties and developed modifications to meet post-thaw storage with CPD-adenine and pH readjustment to 7.2 b) Development of techniques to actually freeze large doses of platelets adaptable to storage in mechanical refrigerators. c) Development of in vitro tests to evaluate platelets, and changes in platelet size distribution due to storage.

* Available to contractors upon additional approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161101A91C	Basic Mechanisms of Recovery From Injury
WORK UNIT NO.	042	Long-Term Cryopreservation of Platelets for Immediate Field Use

The following investigations have been conducted under this work unit:

STUDY NO. 1	Cryopreservation strategies
STUDY NO. 2	In vitro viability function testing

STUDY NOS. 1 and 2. Massive transfusion of stored blood or blood substitutes following severe combat injuries leads to impaired hemostasis due to consumption and dilution of clotting factors and platelets. Platelet transfusions can correct hemostasis problems due to thrombocytopenia. Studies in this division address practical methods whereby platelets can be frozen, stored for long periods, thawed and made ready for immediate or delayed transfusion. This strategy places emphasis on preparing a therapeutic dose, as one unit, with minimal post-thaw manipulation. Toward this goal in vitro work with 4% glycerol and 5% glucose as the cryoprotectant has been done. This procedure allows large dose freezing and takes only 30 minutes post-thaw manipulation. In vitro studies suggest that viable platelets that will fulfill military needs can be maintained by selective cryopreservation procedures. Techniques for evaluating storage capability in vitro have been developed based on shape, size and density alterations of platelets. These techniques will facilitate predictions of subsequent in vivo platelet performance after freezing and storage in the liquid state.

BODY OF REPORT

WORK UNIT NO. 042 Long-term Cryopreservation of Platelets for Immediate Field Use

STUDY NO. 1 Cryopreservation strategies

PROBLEM

Massive transfusion of stored blood or blood substitutes following severe combat injuries leads to impaired hemostasis. This defect aggravates bleeding, leading to an inability to resuscitate the wounded soldier successfully. The defect is due to many factors: trauma, dilution of blood with resuscitation fluids, and the lack of platelets and coagulation factors in stored blood products. Platelets can be prepared and given in massive transfusion situations to prevent and treat bleeding due to thrombocytopenia. Blood and coagulation factors are relatively easy to obtain and stored for massive transfusion needs but platelets stored in conventional liquid storage systems are too perishable (72 hr storage period) for field use. Current freezing schemas for storing platelets are cumbersome and time-consuming. The platelets require extensive post-thaw washing to eliminate possible toxic cryopreservatives and the procedures are not field adaptable. This study is aimed at evaluating simple freezing protocols with field adaptability as well as post-thaw storability out to 72 or more hours. This approach would allow us to adapt current feasible processing of frozen platelets in CONUS locations, then transport as needed to combat zones, and thaw for immediate use. However, if needed in far forward front positions for resuscitation, these post-thaw units would be storable for 3 days or more for convenient shipment and logistical support of forward needs.

RESULTS AND DISCUSSION OF RESULTS

A freezing protocol, based on the work of Drs. Pert and Dayian of Albany, New York, has been established. The cryoprotectant in this protocol is 4% glycerol and 5% glucose. Since these compounds are physiological in the final product (1% less than each glycerol and glucose), the procedure does not require extensive post-thaw processing, it requires only dilution of the platelets with acidified plasma. Tests in our laboratory show this procedure results in a product with acceptable in vitro characteristics. Work with storage in mechanical refrigerators at -80 C was begun. In vitro tests (morphology, sizing, recovery) suggest it is possible to store the product at -80 C instead of -190 C. Studies were performed on the influence of freezing platelets in polyvinyl chloride (PVC) and polyolefin bags. In vitro results suggest PVC is as good as polyolefin. Current procedures using dimethyl sulfoxide as the cryoprotectant require polyolefin bags. Use of polyolefin bags reduces the efficiency of freezing due to bag transfers both before and after freezing. Using the

original donor PVC bags simplifies processing and is more efficient since no transfers are needed. Additional studies on post-thaw liquid storage with adenine supplemented platelet concentrates show a slight improvement in morphology and pH over CPD preserved platelets in vitro. Whether or not adenine will be beneficial needs to be established by in vivo studies.

CONCLUSIONS

The glycerol-glucose protocol for preservation of platelets has characteristics that make it adaptable to military needs. The in vitro studies confirm this approach and suggest practical storage in PVC bags with mechanical refrigeration.

RECOMMENDATIONS

The development of other freezing strategies should be terminated. Efforts should be directed toward practical field adaptability of the glycerol-glucose procedure. In vivo human studies must be done to establish the value of glycerol-glucose preserved platelets. Collaboration should be encouraged with the Pert-Dayian group as well as with the American Red Cross Research Laboratory (Dr. Meryman) to conduct clinical trials and protocols directed to the question of effectiveness.

PUBLICATIONS

None

STUDY NO. 2 In vitro viability function testing

PROBLEM

The development of frozen platelet protocols has had to rely on the ability to evaluate platelets by in vitro parameters. The tests currently available have not been reliable from laboratory to laboratory and have questionable value when the platelets are perturbed by the presence of cryoprotectants.

RESULTS AND DISCUSSION OF RESULTS

We have concentrated our efforts toward developing simple morphological assays of platelet integrity as the basis of our in vitro tests. Electronic sizing, morphology scores, and actual platelet loss by count are the basic tests used in this approach. We have shown that cells become smaller after freezing, regardless of the presence of a cryoprotectant. The shrinkage of the cells appears to reflect storage injury. Morphology scores show similar changes. We feel these tests are invaluable for in

vitro evaluations, however, in vivo correlations are necessary. We have also studied density changes with freezing and find cells become less dense with freezing injury. Although density changes are difficult to measure and therefore impractical for routine use, the technique offers the capability to separate cohorts for identification of various degrees of injury and, possibly, cohorts of nonviable and viable cells. Also, these in vitro observations have been performed on liquid stored platelets to show changes during prolonged storage.

CONCLUSIONS

Techniques to measure changes in size, shape, and density changes are now available to evaluate storage effects on platelets. Measured changes may reflect storage injuries and allow us to predict the subsequent outcome of in vivo recovery, survivability and function of transfused platelets.

RECOMMENDATIONS

The use of in vitro measurements to assess platelet viability in vivo requires the development of human protocols. A human use study in which changes can be correlated to recovery and survival in normal volunteers should be performed before further work is done in which these tests are used to assess cryopreservation protocols. Function tests in which thrombocytopenic patient volunteers participate should also be developed.

PUBLICATIONS

1. BOLIN, R., B. CHENEY, O. SIMPLICIANO, and C.C. PECK. In vitro evaluation of platelets stored in various adenine containing formulations. Transfusion, in press.

2. BOLIN, R., B. CHENEY, O. SIMPLICIANO, and D. SMITH. Density changes in platelet concentrates stored at 22 C. Abstract, American Association of Blood Banks Meeting, Las Vegas, NV, November, 1979.

3. BOLIN, R., B. CHENEY, and F. MEDINA. Glycoprotein changes in density separated platelets during storage at 22 C. Abstract, American Society of Hemotologists, New Orleans, LA, December 1979.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6315	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 23	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	047 APC 504J
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Studies in Behavioral Toxicology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

013400 Psychology; 016200 Stress Physiology; 016800 Toxicology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 12	81 12	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 79	1.0	24
	CURRENT 80	1.0	48

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Hannon, J.P., DAC

NAME: Pribyl, V., DAC POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Behavioral Toxicology; (U) Toxicology; (U) Neurophysiology; (U) Operant Conditioning; (U) Psychopharmacology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Soldiers are routinely exposed to numerous chemical compounds during their performance of military duties. Some of thse compounds are known to produce effects which adversely influence the soldier's ability to perform combat-essential activities with maximum efficiency. In general, however, the behavioral effects of chemical exposure are unknown. The objective of this research is to develop and test techniques for detecting and quantifying changes in sensory and motor capabilities attendant to acute or chronic low-level chemical exposure.

24. (U) Animals are exposed to various concentrations of chemical agents. Behavioral and neurophysiological methods are used to assess initial effects on sensory and motor processes and to monitor recovery following chemical exposure. Biochemical and histopathological techniques are used to provide additional information concerning the biological effects of the chemical agents. The effects of exposure to the chemical components of military munitions and combustion by-products will be studied.

25. (U) (7810-7909). Construction of equipment and preliminary behavioral test evaluation has been completed. Operant training devices have been interfaced to a microprocessor controller. An investigation of diisopropylphosphoroflouridate (DFP) toxicity is in progress. Both acute and delayed neurotoxicity will be studied using this compound. Subsequently, DFP will be used as a positive control during investigations of military munitions compounds.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A161101A91C In-House Laboratory Independent Research

WORK UNIT NO. 047 Studies in Behavioral Toxicology

The following investigation has been conducted under this work unit:

STUDY NO. 1 Development of a rapid screening battery

STUDY NO. 1. The major emphasis in Study No. 1 has been interfacing of operant conditioning modules with a microprocessor controller and construction and preliminary evaluation of a behavioral test battery. Neurophysiological techniques have been developed for assessing sensory effects. An investigation of the acute and chronic effects of diisopropylphosphorofluoridate is in progress. Pilot studies were conducted to determine the feasibility of using the laboratory rat as a model for assessing the effects of chronic low-level exposure to white phosphorus. Physiological, biochemical, and behavioral measurements established the reliability of the model in terms of toxicity levels consistent with the survival and the nature of adaptation to low-level toxicity.

BODY OF REPORT

WORK UNIT NO.	047	Studies in Behavioral Toxicology
STUDY NO.	1	Development of a rapid screening battery

PROBLEM

Soldiers are routinely exposed to numerous chemical compounds during their performance of military duties. Some of these compounds are known to produce effects which adversely influence the soldiers' ability to perform combat-essential activities with maximum efficiency. In general, however, the behavioral effects of chemical exposure are unknown. Proper evaluation of the behavioral test battery requires the use of a chemical which produces known neurotoxic effects. Such a chemical could also be used as a positive control during the investigation of the neurotoxic properties of other compounds.

RESULTS AND DISCUSSION OF RESULTS

In a pilot study, male rats were given a single injection of diisopropylphosphorofluoridate (DFP) mixed with peanut oil in varying doses. When tested three to nine days later on the rotating rod, speed correlated significantly ($p<0.05$) with dose of DFP, $r = -.48$. An investigation of the acute and chronic effects of DFP in male and female rats is in progress. Once a week, rats are injected subcutaneously with a solution of DFP and peanut oil. Tests used to evaluate effects include rotating rod, spontaneous alternation, and rapid avoidance.

CONCLUSIONS

None

RECOMMENDATIONS

Evaluation of the behavioral test battery should be completed. Development of techniques to evaluate sensory impairment should be emphasized. In particular, operant conditioning techniques combined with neurophysiological measures, such as sensory evoked potentials and the electroencephalogram, may provide effective measures of sensory effects of toxic agents.

PUBLICATIONS

LHOTA, J., P.A. O'MARA, and J.P. HANNON. Yellow Phosphorus Toxicity: Pilot Experiments to Detect Alterations in Operant Behavior, Voluntary Activity, and Visual Evoked Potentials. Technical Note No. 79-1TN. San Francisco, California: Letterman Army Institute of Research, 1979

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6316	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 27	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	049 APC 504R
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Toxicological screening of potentially hazardous substances using *Drosophila melanogaster*

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002600 Biology; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 02	80 09	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	79	.3	9
	CURRENT 80	2.0	43

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: Fruin, John T., LTC VC
NAME: Eisenberg, George H.G.,MAJ,MS POC:DA

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Toxicology; (U) Mutagenicity; (U) *Drosophila melanogaster*; (U) Sex-linked recessive lethal test.

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To establish an in-house capability for the toxicological screening of potentially hazardous substances using the *Drosophila melanogaster* sex-linked recessive lethal (SLRL) test.

24. (U) A *D. melanogaster* insectary capable of supporting a SLRL test program will be established, personnel will be trained in SLRL test procedures and the methodology will be developed for the incorporation of test materials into the standard rearing diet.

25. (U) 79 01 - 79 08. The senior investigator has been trained at a leading facility in the development of the SLRL testing procedure, the required *Drosophila* strains have been obtained, all major equipment and supplies have been purchased, and training of technicians in rearing and testing procedures is 60% complete.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT No. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 049 — Toxicological Screening of Potentially Hazardous Substances using *Drosophila melanogaster*

The following investigation has been conducted under this work unit:

STUDY NO. 1 Establishing an in-house capability for the toxicological screening of potentially hazardous substances using the *Drosophila melanogaster* sex-linked recessive lethal test

The Armed Forces are often confronted with unique toxicology problems associated with the varied tasks required for mission completion. While many problems facing the military are unique to its environment, federal requirements must still be met concerning human and environmental exposure to potentially hazardous substances. The Department of Defense does not possess in-house capability for the volume and diversity of compounds that must undergo toxicological testing to meet federal legal requirements. Establishing an in-house capability for the toxicological screening of potentially hazardous substances by using the *Drosophila melanogaster* sex-linked recessive lethal test is part of the LAIR toxicology program designed to help meet these requirements. A Drosophila insectary has been established and personnel are being trained in rearing and testing procedures. All testing will meet the Food and Drug Administration Good Laboratory Practices Regulation.

BODY OF REPORT

WORK UNIT NO.	049	Toxicological Screening of Potentially Hazardous Substances using *Drosophila melanogaster*
STUDY NO.	1	Establishing an in-house capability for the toxicological screening of potentially hazardous substances using the *Drosophila melanogaster* sex-linked recessive lethal test

PROBLEM

Regulations dictate establishment of safety for many new substances proposed for human use. Some of the required tests must be performed before any human contact will be allowed. Other required tests, because they are relatively rapid and inexpensive, are performed at this time to detect unacceptable substances for removal from consideration as soon as possible. The Drosophila mutagenicity test is an example of the latter type of test. After it is established, it is a potent tool for detecting substances that cause genetic disorders. However, it is not a simple procedure to establish, and considerable training is required for proper interpretation of results. As a result, there are few laboratories capable of performing the test. This work unit was initiated to determine the feasibility of establishing and maintaining an in-house capability for performing this test to support Army requirements for toxicological testing.

RESULTS AND DISCUSSION OF RESULTS

The major steps in establishing the capability of conducting sex-linked recessive lethal *Drosophila melanogaster* tests have been completed: the senior investigator has been trained at a lead facility in the development of the procedure, the required Drosophila strains have been obtained, all major equipment and supplies have been purchased, and technicians are being trained in rearing and testing procedures. Standard Operating Procedures are being written for all phases of the test to insure compliance with the Food and Drug Administration Good Laboratory Practice Regulations.

CONCLUSIONS

None

RECOMMENDATIONS

None

Toxicological Screening of Hazardous Substances using *D.melanogaster*

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 0178	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 15	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	050 APC 506E
b. CONTRIBUTING				
c. CONTRIBUTING	None			

11. TITLE (Procede with security Classification Code)

(U) Toxicology of Explosives and Explosive By-Products

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

0016800 Toxicology; 005900 Environmental Biology; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 07		DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	0.4	50
	CURRENT 80	6.0	107

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

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ASSOCIATE INVESTIGATORS POC: DA

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Military Toxicology; (U) Munitions Chemicals; (U) Carcinogenesis; (U) Teratogenesis; (U) Military Performance

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security Classification Code.)

23. (U) Under provisions of the National Policy Act of 1969, and all other federal Environmental laws, the U.S. Army is assigned responsibility for the protection of soldiers during training and combat from chemicals generated by military activities, and the protection of the civilian community from chemicals generated by military industrial or other activities. Because of the markedly increasing requirements for toxicology testing by industry and government agencies and a critical national shortage of facilities and trained personnel to address these requirements, the U.S. Army faces an untenable position in discharging its assigned responsibilities. The purpose of this work unit, therefore, is to establish and implement an in-house toxicology program specifically directed to the test and evaluation of environmental chemical contaminants generated by munitions manufacture and use.

24. (U) Two areas of research will be pursued. The first will be concerned with the test and evaluation of candidate chemicals for pathologic, mutagenic, carcinogenic, reproductive or teratogenic effects that may pose a health hazard to humans. The second research area will be concerned with the impact of candidate chemicals on combat-related performance factors and the evaluation of treatment modalities when adverse effects are observed.

25. (U) 7810-7909. Protocols and Standard Operating Procedures for studying the acute and subacute toxicity of 2,4 dinitrotoluene (2,4 DNT) have been written and approved. The purity of the test compound has been assayed. Initial studies are being implemented.

*Available in contractor's own exhibitor's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A161101A91C Military Toxicology

WORK UNIT NO. 050 Toxicology of Explosives and Explosive By-Products

The following investigation has been conducted under this work unit:

STUDY NO. 1 Toxicology of 2,4-dinitrotoluene (2,4-DNT)

STUDY NO. 1 The National Policy Act of 1969 and other Federal environmental laws assign to the U.S. Army the responsibility for protection of soldiers during training and combat from chemicals generated by military activities, and the protection of the civilian community from chemicals generated by military, industrial, or other activities. Exposure to potentially hazardous chemicals may occur among workers employed in munitions plants, to the civilian population in the vicinity of the plant as the result of environmental contamination associated with munitions manufacture and assembly, and to soldiers who use the munitions in training or combat. The U.S. Army Medical Research and Development Command directed the Letterman Army Institute of Research to develop a capability in toxicology research and to determine the maximum tolerated dose (MTD) of 2,4-DNT that may be given to animals during feeding studies over a 24-month period. Protocols and standing operating procedures have been prepared and approved. The purity of the test compound has been assayed and initial studies to determine the approximate lethal dose and median lethal dose (LD_{50}) of 2,4-DNT in rats are being initiated.

BODY OF REPORT

WORK UNIT NO. 050 Military Toxicology

STUDY NO. 1 Toxicology of 2,4-dinitrotoluene (2,4-DNT)

PROBLEM

Exposure to chemicals associated with the manufacture and assembly of military munitions may constitute a health hazard to workers in munitions plants, the civilian population in the vicinity of the plant as a result of environmental contamination, and soldiers who use munitions in training and combat. The National Policy Act of 1969 and other Federal environmental laws assign the responsibility for protection of soldiers, munitions plant workers, and the civilian population to the U.S. Army. The U.S. Army Medical Research and Development Command has directed the Letterman Army Institute of Research to develop a capability for toxicologic testing of chemicals in animals and specifically to conduct acute, subacute, and subchronic toxicology studies on chemical by-products of the munitions industry. A major area of concern at the present time is the toxicologic effects of 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitrohexahydro-1,3,5 triazine (RDX) and their by-products. These chemicals from shells with TNT and RDX mixtures are discharged into the environment without significant treatment to the waste waters. The waste waters are commonly referred to as LAP (load, assemble, and park) waters which contain a 1.6:1 blend of TNT:RDX and condensate water which contains approximately thirty compounds produced by irradiation (sunlight) of TNT-RDX mixtures.

This study is concerned with the acute, subacute, and subchronic toxicity of 2,4-dinitrotoluene (2,4-DNT), a major component (approximately 43% relative concentration) of condensate water. Other government-supported studies have addressed this subject. The available toxicologic data, however, do not fully satisfy requirements for assessment of long-term human health hazards, or the establishment of comprehensive environmental standards for this compound. Thus, there is a need for verification of earlier findings.

The overall purpose of this study is to determine the maximum tolerated dose (MTD) of 2,4-DNT that may be given to rats and mice during a 24-month chronic feeding study. Specific objectives include the determination of LD_{50} for both species, identification of target organs, dose-response relationships during and following a continuous 14-day subacute feeding, and description of the toxic effects produced by subchronic (90-day) feeding at various dose levels (from low doses to the highest dose approximating the MTD).

Military toxicology (cont'd)

RESULTS AND DISCUSSION OF RESULTS

This is a new work unit and no data are available as yet. Since toxicologic testing has many requirements which differ from the requirements of previous research conducted at LAIR, many administrative changes, much procedural development, a great deal of technical training have been required before actual studies could be initiated. Protocols and standing operating procedures have been written, revised, and adopted. Since this study and future studies of this type are to be conducted in compliance with procedures outlined in the Good Laboratory Practices Act required by the Food and Drug Administration (FDA), and with similar procedures proposed by the Environmental Protection Agency (EPA), a number of modifications of administrative and technical laboratory procedures remain to be done. The test compound has been obtained and assays for its purity have been performed. Initial studies to determine the median lethal dose (LD_{50}) have been initiated.

CONCLUSIONS

None

RECOMMENDATIONS

This study should be completed and a capability to perform toxicologic studies in compliance with FDA and EPA regulations should be developed and refined.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6079	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	D. Change	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3A162772A813	00	021 APC 504L
b. CONTRIBUTING	61102B	3E161102BS01	00	202
c. CONTRIBUTING	CARDS 114f			

11. TITLE (Procede with Security Classification Code):

(U) Determination of Threshold Data from Coherent and Incoherent Radiation Sources

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

09600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 12	Cont	DA	C. In-House

17. CONTRACT GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	3.0	89
	CURRENT 80	3.0	76

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: Stuck, B.E., DAC

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Eye Protection; (U) Infrared Lasers; (U) Systems Safety; (U) Laser Hazard; (U) Eye Damage; (U) Skin Damage

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objectives are to experimentally determine dose response relationships for infrared laser radiation for exposure conditions relevant to Army laser systems operation and to recommend permissible exposure limits based upon these bioeffects data.

24. (U) The ED_{50} (effective dose required to produce a specified response 50% of the time) for various exposure conditions and response criteria are determined. Cornea effects are evaluated at various time intervals by direct observation, histological techniques, and specular microscopy on Rhesus monkey eyes.

25. (U) (7810-7909). The corneal ED_{50}'s were determined for long pulse and Q-switched holmium laser radiation at 2.06μ and long pulse erbium laser at 1.54μ. These results and exposure conditions are summarized as follows: 1) Holmium laser, long pulse at 2.06μ, exposure duration 100 microseconds (FWHM), ED_{50} = 2.9J/cm^2, effective irradiance diameter 1.8 mm, dose range tested 0.6-6.2 J/cm^2, 2) Holmium laser, Q-switched at 2.06μ, exposure duration 43 microseconds (FWHM), ED_{50} = 5 J/cm^2, effective irradiance diameter of 0.32 mm, dose range tested 1.0 to 19 J/cm^2, 3) Erbium laser radiation at 1.54μ, exposure duration 930 microseconds, ED_{50} = 9.6 J/cm^2, effective beam diameter 1.0 mm dose range tested from 1.4-22 J/cm^2. The corneal depth response was determined and correlated with the absorption characteristics of the cornea. These results indicate a distinct wavelength dependence of the corneal damage threshold for infrared laser radiation. No visible alterations to other ocular structures (i.e., lens, retina) were seen.

Two Rhesus monkeys were exposed to a large field of diffuse argon laser radiation at 514.5 nm for two-hour periods with a screen radiance of 6.1 uw/cm^{2}sr. A total of 20 hours of exposure was accumulated over a three-week period before the subjects were sacrificed for retinal evaluation by light and electron microscopy. Retinal sections from these subjects are currently being evaluated.

*Available to contractors upon originator's approval.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3E161102BS01	Basic Research on Military Injury and Disease
WORK UNIT NO.	021	Determination of Threshold Data from Coherent and Incoherent Radiation Sources

The following investigation has been conducted under this work unit:

STUDY NO. 1 Ocular and skin effects of infrared laser radiation

STUDY NO. 1. Corneal dose reponse relationships were determined for long pulse erbium laser radiation at 1.54 μ and long pulse and Q-switched holmium laser radiation at 2.06 μ. These results and exposure conditions are summarized as follows: 1) holmium laser, long pulse at 2.06 μ, exposure duration 100 microseconds (FWHM), ED_{50} = 2.9 J/cm^2, effective irradiance diameter 1.8 mm, dose range tested 0.6-6.2 J/cm^2; 2) holmium laser, Q-switched at 2.06 μ, exposure duration 43 microseconds (FWHM), ED_{50} = 5 J/cm^2, effective irradiance diameter of 0.32 mm, dose range tested 1.0 to 19 J/cm^2; 3) erbium laser radiation at 1.54 μ, exposure duration 930 microseconds, ED_{50} = 9.6 J/cm^2, effective beam diameter 1.0 mm, dose range tested from 1.4-22 J/cm^2. The depth of the corneal response was determined and correlated with the absorption characteristics of the cornea. These results indicate a distinct wavelength dependence of the corneal damage threshold for infrared laser radiation. No visible alterations to other ocular structures (i.e., lens, retina) were seen.

Two Rhesus monkeys were exposed to a large field of diffuse argon laser radiation at 514.5 nm for two-hour periods with a screen radiance of 6.1 μw/cm^{2}sr. A total of 20 hours of exposure was accumulated over a three-week period before the subjects were sacrificed for retinal evaluation by light and electron microscopy. Retinal sections from these subjects are currently being evaluated.

BODY OF REPORT

WORK UNIT NO.	021	Determination of Threshold Data from Coherent and Incoherent Radiation Sources
STUDY NO.	1	Ocular and skin effects of infrared laser radiation

PROBLEM

Current and proposed military laser systems operate in the infrared region beyond 1.4 microns. In the spectral region from 1.4 to 3.0 μ, the absorption coefficients of the outer ocular media (cornea, aqueous, lens, and vitreous) vary over three orders magnitude. Although limited data are available for specific exposure conditions, the wavelength dependence of the dose-response relationships relevant to Army systems has not been adequately defined. Permissible exposure limits have been defined in TB MED 279; however, bioeffects data for exposure conditions in this spectral region may warrant change in permissible exposure limits and impact on the design and employment of military systems.

In previous work conducted under Work Unit No. 025, Rhesus monkey spectral sensitivity for fine resolution criteria was permanently altered after repeated low-level exposure to diffuse argon laser radiation. These exposure conditions are comparable to those anticipated in the use of laser scanned visual displays. Research was initiated to determine if a morphological correlate to the functional alteration was apparent by light and electron microscopic evaluation.

RESULTS AND DISCUSSION OF RESULTS

Corneal dose response relationships were determined for long pulse erbium laser radiation at 1.54 μ and long pulse and Q-switched holmium laser radiation at 2.06 μ. These results and exposure conditions are summarized as follows: 1) holmium laser, long pulse at 2.06 μ, exposure duration 100 microseconds (FWHM), ED_{50} = 2.9 J/cm^2, effective irradiance diameter 1.8 mm, dose range tested 0.6-6.2 J/cm^2; 2) holmium laser, Q-switched at 2.06 μ, exposure duration 43 microseconds (FWHM), ED_{50} = 5 J/cm^2, effective irradiance diameter of 0.32 mm, dose range tested 1.0 to 19 J/cm^2; 3) erbium laser radiation at 1.54 μ, exposure duration 930 microseconds, ED_{50} = 9.6 J/cm^2, effective beam diameter 1.0 mm, dose range tested from 1.4-22 J/cm^2.

The ocular response for these exposure conditions was confined to the cornea as observed by slit lamp microscopy. No lesions were observed

at 24 hours or subsequent observation sessions that had not been observed at one hour. The depth of the lesions and the diameter of the lesions were both dose and wavelength dependent. For both holmium and erbium laser exposures, the corneal opacity (i.e., residual stromal scar) persisted for periods at least to 10 months (last observation). Lesions produced by erbium laser exposures were deeper and changed little over a 10-month period. Although some holmium laser-induced lesions visible at one hour produced by holmium laser exposures were not observable at 24-48 hours, most persisted. The depth of the response extended through Bowman's membrane. The persistence of the response is indicative of alteration of the stroma which does not undergo rapid rapair. Changes in lesion appearance that did occur in the first 24-48 hour period were attributed to regeneration or "clearing" of the corneal epithelium. The steep slopes of the dose-response curves are indicative of the small increase in dose required for response to vary from no observed effect to a high probability of observing an effect. Repeated exposures to doses 10-50 times the current maximum permissible exposure for these conditions resulted in no corneal change. Corneal endothelial alterations determined by in vivo specular microscopy occurred only at doses which produced stromal scars.

In previous work, Rhesus monkey spectral sensitivity for fine resolution criteria was permanently altered after repeated low-level exposure to diffuse argon laser radiation. In this parallel histopathology study, two untrained Rhesus monkeys (3-4 years old) were exposed under similar conditions and their retinas were evaluated by light and electron microscopy. The chaired subject's head was restrained and positioned near the center of 0.75 m hemisphere which was diffusely irradiated with an argon laser operating at 514.5 nm. The radiance of the hemisphere was 6 $\mu w/cm^2/sr$ and the subjects were exposed for two hours in each session. The first subject received 22 cumulative hours of binocular exposure in 11 sessions over a 15-day period. The second subject was monocularly exposed for 24 cumulative hours in 12 sessions over a 25-day period. The measured pupil diameter during exposure was between 4.0 and 4.4 mm for both subjects. The eyes were enucleated four days after the last exposure sessions. Notable observed changes were restricted to the photoreceptors and the pigment epithelium. These changes include swollen tortuous photoreceptor outer segments and some disorganized lamellae surrounded by increased subretinal fluid. Different degrees of mitochondrial changes in adjacent photoreceptor inner segments were observed in some cases. While changes were observed, few quantitative differences are presently discernible between the patched eye and the exposed eyes. At present, a morphological correlate to spectral sensitivity alteration is not yet evident for these exposure conditions and time of enucleation.

Determination Threshold Data Coherent/Incoherent Radiation Sources (Cont)

CONCLUSIONS

These are interrelated with the stated Recommendations.

RECOMMENDATIONS

Although additional experimental data are needed for long exposure durations and larger corneal irradiance diameters for infrared laser exposures from 1.4 μ to 3.0 μ, a generalized wavelength correction to current permissible exposures appears necessary based upon the relative absorption properties of the ocular media.

PUBLICATIONS

None

PRESENTATIONS

1. BEATRICE, E.S., and B.E. STUCK. Current Army laser bioeffects research. Presented at the NEI-NRC Workshop on Ocular Safety and Eye Care at Duke University Medical Center (Durham, North Carolina, October 1978)

2. STUCK, B.E. Q-switched and PRF lasers. Presented at DOD RDT&E Topical Review on Bioeffects of Non-Ionizing Radiation at Naval Medical Research Institute (Bethesda, Maryland, June 1979)

3. BEATRICE, E.S. 1) Chronic (repeated) light exposure and 2) Future research requirements and policy constraints. Presented by B.E. Stuck at DOD RDT&E Topical Review on Bioeffects of Non-Ionizing Radiation at Naval Medical Research Institute (Bethesda, Maryland, June 1979)

4. STUCK, B.E. Corneal effects of erbium and holmium laser radiation. Presented at Tri-Service Laser Bioeffects Coordination Meeting at LAIR (San Francisco, California, March 1979)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6308	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3A162772A813	00	022 APC 504L
b. CONTRIBUTING	61102B	3E161102BS01	00	205
c. CONTRIBUTING	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) System Developer Assistance Studies in Laser Bioeffects

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

009600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 07	Cont	DA	C. In-House

17. CONTRACT GRANT

a. DATES/EFFECTIVE — EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE — d. AMOUNT:

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	3.0	125
FISCAL YEAR CURRENT 80	1.0	41

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Erbium; (U) Repetitive Pulse; (U) Ocular Hazard; (U) Damage Threshold; (U) Laser Safety; (U) GaAs; (U) Neodymium

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To provide bioeffect data base for safety documentation of laser training devices and to improve the accuracy of safety standards as applied to laser training devices. To evaluate the occular hazard of near infrared lasers considered for future laser training devices.

24. (U) Evaluate retinal tissue exposed to the MILES laser transmitter via electron microscopy. Determine ED_{50} versus retinal irradiation diameter for 1 μsec exposure duration. Determine the ED_{50} for near infrared lasers.

25. (U) (7810-7909). Rhesus monkey retinae were subjected to irradiation from a MILES M-16 GaAs laser transmitter. The tissue was prepared for electron microscopy and submitted to three experts for evaluation. The analysis has not yet been returned. The ED_{50} for retinal burn via dia laser was determined as a function of retinal irradiation diameter. The pulse duration was 400 μsec. The ED_{50}'s were: minimum spot, 5.2 μJ; 400 μ spot, 55 μJ; 1000 μ spot, 143 μJ. The ED_{50} for ocular damage via 1.33 μ Nd laser was determined. The exposure duration was 5 sec. The damage site was the cornea with ED_{50} of 43 w/cm^2. The effective beam diameter was 1.5 mm. The range of doses was 12 to 87 w/cm^2.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3E161102BS01 Basic Research on Military Injury and Disease

WORK UNIT NO. 022 System Developer Assistnace Studies in Laser Bioeffects

The following investigations have been conducted under this work unit:

STUDY NO. 1 Project MILES

STUDY NO. 3 Electrophysiological evaluation of retinal alterations following laser irradiation

STUDY NO. 1. Rhesus monkey retinae were subjected to irradiation from a MILES M-16 GaAs laser transmitter. The tissue was prepared for electron microscopy and submitted to an expert for evaluation. The analysis has not yet been returned.

Retinal exposures were made in Rhesus monkey with a 120 kHz GaAs laser. Doses ranged from well above to well below the ED_{50} for retinal burn. No retinal clouding was observed. Spectral photography of the exposed retina was performed and evaluated.

ED_{50} for retinal burn via dye laser was determined as a function of retinal irradiation diameter. The pulse duration was 400 μs. The ED_{50}s were: minimum spot, 5.2 μJ; 400 μ spot, 55 μJ; 1000 μ spot, 143 μJ.

The ED_{50} for ocular damage via 1.33 μ Nd laser was determined. The exposure duration was 5 s. The damage site was the cornea with ED_{50} of 43 w/cm^2. The effective beam diameter was 1.5 mm. The range of doses was 12 to 87 w/cm^2.

STUDY NO. 3. A technique has been developed for evaluating the immediate short and long-term effects of laser radiation upon the visual system of the Rhesus monkey. A grating was attached to a linear motion motor and mounted in a fundus camera. The image of the grating was focused onto the retina of curarized Rhesus monkeys and visual evoked cortical potentials (VECPs) were obtained by shifting the grating one bar width left or right. The resultant VECPs were reproducible for as few as eight simulated potentials. The latency of the VECP following argon and gallium arsenide laser exposures were significantly different.

BODY OF REPORT

WORK UNIT NO. 022 System Developer Assistance Studies in Laser Bioeffects

STUDY NO. 1 Project MILES

PROBLEM

Military training devices using GaAs laser transmitters are widely deployed within the Army. Use of these devices requires intentional exposure of personnel to laser irradiation. It is essential that the ocular hazard of lasers used in these training devices be completely understood.

RESULTS AND DISCUSSION OF RESULTS

In an effort to understand the phenomena of "retinal clouding" produced by low dose GaAs laser exposure, exposed retinal tissue was submitted to Dr. Kuwabara of the National Eye Institute, Bethesda, Maryland, for electron microscopic analysis. The analysis has not yet been returned.

A pulsed GaAs laser operating at a repetition rate of 120 kHz was incorporated into a delivery system for retinal exposure. Retinal exposures in Rhesus monkey were performed at durations of 1 s and 30 s. Doses ranged from 2 x ED_{50} to $ED_{50}/3$. No retinal clouding was seen at the lower doses. The exposed retinae were photographed through narrow bandpass filters centered at several wavelengths ranging from 300 nm to 900 nm. Exposure sites exposed at the ED_{50} dose could be detected as darkened areas in the long wavelength (> 700 nm) spectral photographs. These sites were not differentiated at the shorter wavelengths.

The ED_{50} as a function of retinal irradiation area was determined for a pulsed dye laser. The laser emitted 400 ns pulses at 600 nm. Thermal damage models predict that the ED_{50} dose will be linearly proportional to the irradiated area for pulse durations near 1 μs. The dye laser data do not support this prediction. The ED_{50}s were: minimal spot, 5.2 μJ; 400 μ spot, 55 μJ; 1000 μ spot, 143 μJ. These data follow the relationship ED_{50} = (retinal area)$^{\frac{1}{2}}$, which is consistent with the data for exposure durations both longer than and shorter than 1 μs.

A cw Nd:YAG laser was modified to emit 1.318 μ and 1.338 μ radiation. Rhesus monkey eyes were subjected to irradiation of 5 s duration. The maximum dose was 2 watts. No retinal alterations were observed. The ED_{50} for corneal damage was 43 w/cm^2 when the irradiated area was

1.5 mm. These data are consistent with the absorption characteristics of the ocular media.

CONCLUSIONS

None

RECOMMENDATIONS

Further studies in these areas are required. Complete understanding of the ocular effects of 1.3 micron irradiation is essential because it potentially offers a relatively safe laser made from a well-developed material.

PUBLICATIONS

LUND, D.J., and E.S. BEATRICE. Ocular hazard of short pulse argon laser irradiation. Health Physics 36:7-11, 1979

PRESENTATIONS

1. LUND, D.J. Ocular Effects of GaAs Lasers. Presented at Tri-Service Coordinated Meeting on Laser Bioeffects, LAIR (San Francisco, California, March 1979)

2. LUND, D.J., and E.S. BEATRICE. Spectral photography of retinal lesions. Presented at Association for Research in Vision and Ophthalmology Annual Meeting (Sarasota, Florida, April 1979)

3. LUND, D.J. Ocular effects of laser training devices. Presented at DOD RDT&E Topical Review, Naval Medical Research Institute (Bethesda, Maryland, June 1979)

STUDY NO. 3. Electrophysiological evaluation of retinal alterations following laser irradiation

PROBLEM

The increased use of lasers in the battlefield has raised many questions about the biological effects of this radiation upon the ability of soldiers to perform their assigned tasks. The effects of high-level laser irradiation of the retina have been studied in the Rhesus monkey. The functional effects of low-level subophthalmoscopically visible laser irradiation of the retina have been difficult to evaluate. Animal training techniques have proven long and costly, while electron microscopy and other intrusive techniques

do not allow for long-term follow-up of the functional effects of laser and other intense light sources.

RESULTS AND DISCUSSION OF RESULTS

The electrophysiological evaluation of the visual cortex following laser irradiation of the eye has made considerable progress. The incorporation of the linear motion motor into the optics of a fundus camera has enabled us to project accurately a grating onto the Rhesus monkey retina. This technique allowed us to require fewer visual evoked cortical potentials (VECPs) than would usually be the case (8 vs 64) to reproduce the waveform reliably. Several studies have been accomplished. 1) The latency of several components of the VECP became significantly more variable immediately following a 60 s, 16 mJ gallium arsenide (905 nm) laser exposure to the animal's foveal area. The animal's responses were analyzed in eight-second blocks (one sweep per second) and recovery--the return of latency to within normal limits--occurred approximately 110 s after exposure. 2) An argon laser exposure was then made in the animal's other eye. A 500 μJ, 16 ms pulse of 514 nm laser radiation was directed to the fovea. An immediate change in the latency of a major component of the VECP was noted. This time, however, no evidence of recovery (return to normal latency) was observed, up to 2 h after exposure. Two days later, the latency appeared normal, as it did in subsequent recording sessions. 3) A significant difference was observed in the VECPs when the grating was shifted from left to right as opposed to right to left movement. These data are currently being analyzed to determine the relationship between the direction of movement and the frequency at which the VECPs are elicited.

CONCLUSIONS

Analysis of visual evoked cortical response waveforms can be useful in determining that changes have occurred in the visual system following ocular laser exposures.

RECOMMENDATIONS

Followup of the previously exposed animals is necessary to determine the long-term effects of laser radiation upon the visual functions of the Rhesus. Correlative studies are needed to relate elements of the VECP directly with known functional parameters, such as acuity and color vision.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6102	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3A162772A813	00	023 APC 504J
b. [redacted]	61102B	3E161102BS01	00	203
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Military Stress and Combat Effectiveness

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

013400 Psychology; 005900 Environmental Biology; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
75 08	Cont	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 79	3.0	100
	CURRENT 80	4.0	91

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Stamper, D.A., DAC

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Military Performance; (U) Human Performance; (U) Visual Tracking; (U) Psychological

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The severe stress encountered in warfare may influence the soldier's ability to perform combat essential activities with maximum efficiency. The objectives of this research are to study 1) weapons effects on the performance of military tasks, 2) weapons systems environments and combat effectiveness, and 3) biomedical factors limiting soldier effectiveness. Research is conducted under field conditions and in the laboratory.

24. (U) Animals or human subjects are subjected to conditions which produce stress of varying intensity and durations. The effects of stressors are confirmed biochemically and through observation of physiological and psychological indices. Experimental stress is then related to the ability of subjects to perform various tasks. For human subjects, target acquisition and tracking, communications, endurance, and vigilance tasks are employed. Operant techniques are used with animal subjects.

25. (U) (7810-7909). A field simulation laboratory has been constructed which will facilitate the controlled investigation of biomedical factors which may influence laser designator operations through their effects on the individual. The laboratory includes a sandbag bunker housing a laser designator, a terrain model, and scale model remote control targets. Automatic data acquisition and computer control of experimental conditions are employed. The results thus far have provided data concerning the increased amount of tracking error experienced as velocity of the target increases and as the amount of ambient light decreases. Additionally, estimates of the amount of time required to train the laser-designator operators are suggested by this data.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3E161102BS01 — Basic Research on Military Injury and Disease

WORK UNIT NO. 023 — Military Stress and Combat Effectiveness

The following investigations have been conducted under this work unit:

STUDY NO. 6 Biomedical factors affecting laser-designator operator performance

EX-1 Evaluation of the effects of target speed and ambient lighting conditions; validation of the Project BLASER

EX-2 Countermeasures directed against laser-designator operators; high intensity quasi-monochromatic flashes

EX-3 Physiological changes associated with laser-designator operator performance

EX-1. Evaluation of root mean square (RMS) error tracking scores under high ambient lighting conditions that were collected by use of the BLASER simulator compared favorably with previous field experiments where several of the military laser-designator devices were used. Further validity for the BLASER tracking data was found as RMS error scores significantly increased as target angular velocity increased and as ambient light levels decreased. These data support continued use of the BLASER simulator to provide tracking data that can assist in developing training programs for laser-designator operators.

EX-2. Thus far, data collection phase of the experiment performed to evaluate the possible disruptive effects of a single quasi-monochromatic flash introduced into the optics of a laser-designator device has been completed. The analysis of the data has begun and will be submitted as a LAIR Institute Report.

EX-3. Thus far, none of the physiological variables included in this type protocol have been used. However, the preliminary indications from the flash experiment (EX-2) suggest that several of these variables which have been reported to be associated with increased levels of stress could provide useful information in several of the upcoming experiments.

BODY OF REPORT

WORK UNIT NO.	023	Military Stress and Combat Effectiveness
STUDY NO.	6	Biomedical factors affecting laser-designator operator performance
EX-1		Evaluation of the effects of target speed and ambient lighting conditions; validation of the Project BLASER

PROBLEM

Laser rangefinder-designator devices and laser weapon systems will play an important role in future military operations. The increased use of these laser devices has led to the study of the biomedical factors related to laser operations. This laboratory has constructed a simulator to study the effects of psychological stress, environmental factors, and antipersonnel countermeasures on laser-designator operator performance.

RESULTS AND DISCUSSION OF RESULTS

From the first experiment, two in-house reports have been prepared. The first report (O'Mara et al., 1979) presents a description of the physical characteristics of the BLASER simulator. Additionally, some preliminary tracking data which describe the shape of the learning curve through ninety 10 s trials are discussed. In the second report (Stamper et al., 1980), the effects of varied target angular velocity and ambient lighting condition on the operator's ability to track accurately a moving target are discussed. It was found that as target velocity increased from 2.5 mrad/s and as ambient light levels decrease from a bright light condition to a dim light condition, significant performance decrements can be expected. The aiming error data correspond well with previous field study data which used several different laser weapon viscous-damped tracking systems.

CONCLUSIONS

The BLASER simulator can be used to provide information concerning how individual and environmental factors can affect tracking performance.

RECOMMENDATIONS

None

PUBLICATIONS

1. O'MARA, P.A., D.A. STAMPER, E. BEATRICE, D.J. LUND, R.L. JONES, R. SERENBETZ, and J.P. HANNON. BLASER: A Simulator for the Investigation of Biomedical Factors Influencing Laser Designator Operator Performance. Technical Note No. 79-10TN. San Francisco, California: Letterman Army Institute of Research, July 1979

2. STAMPER, D.A., P.A. O'MARA, E. BEATRICE, and D.J. LUND. Tracking Performance with a Viscous-Damped Mount under Simulated Conditions of Varied Ambient Light Levels and Target Velocities. Institute Report No. 82. San Francisco, California: Letterman Army Institute of Research, January 1980

EX-2 Countermeasures directed against laser-designator operators; high intensity quasi-monochromatic flashes

PROBLEM

Laser rangefinder-designator devices and laser weapon systems will play an important role in future military operations. The increased use of these laser devices has led to the study of the biomedical factors related to laser operations. This laboratory has constructed a simulator to study the effects of psychological stress, environmental factors, and antipersonnel countermeasures on laser-designator operator performance.

RESULTS AND DISCUSSION OF RESULTS

This experiment, which has just been concluded, evaluated the effects of a single quasi-monochromatic flash that was introduced into the optics of the laser designator while the operator was tracking a moving target under bright light and dim ambient light conditions. The analysis of these data has begun and the results will be submitted in the form of an Institute Report soon.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

EX-3 Physiological changes associated with laser-designator operator performance

PROBLEM

Laser rangefinder-designator devices and laser weapon systems will play an important role in future military operations. The increased use of these laser devices has led to the study of the biomedical factors related to laser operations. This laboratory has constructed a simulator to study the effects of psychological stress, environmental factors, and antipersonnel countermeasures on laser-designator operator performance.

RESULTS AND DISCUSSION OF RESULTS

Thus far, none of the physiologic measures have been used. However, reports from a study just completed which used a quasi-monochromatic flash suggested that stress measures such as increased heart rate and galvanic skin response could provide valuable information in upcoming studies of a similar nature.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6078	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	D. Change	U	U	NA	NL	☑ YES ☐ NO	A. WORK UNIT

10. NO 'CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3A162772A813	00	025 APC 504L
b. CONTRIBUTING	61102B	3E161102BS01	00	281
c. CONTRIBUTING	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Biological Investigations in Prediction and Protection Against Coherent Radiation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

009600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 12	Cont	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	4.1	258
	CURRENT 80	8.0	363

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Randolph, D.I, DAC POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede each with Security Classification Code)

(U) Laser Systems Safety; (U) Laser Hazard; (U) Eye Damage; (U) Vision

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine low level laser effects on visual process that may exist at levels many times below those levels currently specified as "safe" by present laser safety standards. Such information is used by USAEHA to develop Army laser safety standards most reflective of current research and by the DARCOM system developer for use in design of new laser systems.

24. (U) Measures of visual acuity, color vision, contrast sensitivity, dynamic acuity, visual electrophysiology are employed. Permanent change in such measurements for repeated low level exposure are investigated and compared with present levels considered eye safe.

25. (U) (7810-7909). 1. Measures of rhesus visual acuity and spectral sensitivity continue to show permanent loss of visual function after exposure to levels of argon laser (514.5 nm) more than 100 times below safe level for extended source viewing. 2. Extracellular single cell recording in lower vertebrate species indicate that comparable levels and exposure regimes can alter permanently basic visual process as evidenced by changes in spectral sensitivity and receptive field organization. 3. Correlary morphology investigations of low level argon exposure has been initiated and data from several animals is now being evaluated. 4. Visual evoked potentials using alternating bar patterns and spectral dynamic acuity procedures have been established for further study of laser effects on vision. 5. Specialized human visual tests for measurement of macular and peripheral function have been developed employing LED light sources and small microcomputer for rapid manipulation of test parameters. Such tests can be employed to monitor the vision of military personnel closely associated with low level chronic laser exposure conditions.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3E161102BS01 Basic Research on Military Injury and Disease

WORK UNIT NO. 025 Biological Investigations in Prediction and Protection against Coherent Radiation

The following investigation has been conducted under this work unit:

STUDY NO. 1 Effects of laser irradiation on visual function

STUDY NO. 1. Low-level laser exposure in non-human primates (Rhesus) produced changes in visual function at doses many times below extended source permissible exposure criteria. These behavioral effects have been followed over 30 months with evidence of slow decline in remaining central vision. Two Rhesus monkeys have so far been used in this study. Parallel electrophysiological investigations in other Rhesus monkeys showed similar results at higher retinal irradiance levels but still below minimal permissible exposure criteria. In vertebrate subjects, electrophysiological experiments were done which indicate that coherency and associated physical phenomena are involved in producing low-level laser light effects. More refined behavioral and neurophysiological assessment techniques have been developed this year to resolve the nature of the damage mechanisms involved. Collaboration on relevance of these findings to laser safety, as well as work in developing more suitable visual assessment criteria for humans, is in progress.

BODY OF REPORT

WORK UNIT NO.	025	Biological Investigations in Prediction and Protection against Coherent Radiation
STUDY NO.	1	Effects of laser irradiation on visual function

PROBLEM

Current military laser safety standards have been developed for the acute exposure situation where ocular damage is immediately apparent. Standards developed for acute exposure, however, may not be extrapolatable to those situations where viewing of laser light is task related. For example, new concepts in military training simulation involve direct use of low-level laser light in reproducing terrain features and mission engagement scenarios not possible with conventional simulators. Other training situations, military laser holography, and in general low-level repetitive exposure situations are also examples of situations for which acute laser biomedical data are inadequate. Behavioral and electrophysiological assessment of such laser exposure paradigms have been examined. Extrapolation of such data to human laser safety standards is the major objective of this research.

RESULTS AND DISCUSSION

Behavioral experiments in which Rhesus monkeys were chronically exposed to low-level argon (514.5 nm) diffuse laser light have shown that changes in visual function can be produced at levels of exposure well below those that would be predicted from acute exposure laser safety standards. In both behavioral and electrophysiological experiments, these effects were generally not immediately observable. They usually require repeated exposure for full effect to occur. In both behavioral and various electrophysiological experiments, progressive effects were often observed after exposure had ceased. Changes in Rhesus spectral sensitivity up to three years after exposure were observed. In vertebrate electrophysiological investigation, direct evidence was obtained that the coherency of a laser source can uniquely affect the retinal photoreceptor processes.

In related work, a program has been established in conjunction with the Army Environmental Hygiene Agency (AEHA) to develop human visual test apparatus capable of measuring subtle change in human visual function that may result from chronic laser exposure. A dark adaptation device has been instrumented at LAIR. Development of several other techniques involving spatial vision and techniques for measuring human retinal function is planned.

CONCLUSIONS

None

RECOMMENDATIONS

Behavioral investigation should be expanded by training additional animals. Three additional animals are presently being trained. Multiline laser low-level chronic effects should be assessed in additional animals. Such effects will be relevant to full "color" laser training simulators now planned. Investigations should be continued with neurophysiological techniques to determine more adequately damage mechanism effects to neural processing incurred at low levels of laser irradiation. New techniques should be developed to assess the "behavioral" lesion. Electron microscopy and other morphological techniques may be required. When possible, the results of these experiments should be extrapolated to present laser safety standards and to develop general guidelines for using lasers in proposed training situations. The development of adequate visual assessment tools that can be used rapidly to assess human vision may be of extreme importance in the routine use of such systems.

PUBLICATIONS

Publications reflecting the research of Work Unit No. 025 are listed in the Annual Research Progress Report, Letterman Army Institute of Research, 30 September 1978, pp 318-319.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6377	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORG'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	055 APC 505A
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS 1141			

11. TITLE (Precede with Security Classification Code)

(U) Design and Support of Military Biomedical Research Information Systems (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

00420 Computers; 009700 Mathematics and Statistics

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	3.0	128
	CURRENT 80	10.0	260

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Digital Computers; (U) Data Base Managment; (U) Data Files; (U) Biomedical Research Information; (U) Statistics

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective is to design, implement, and document computer programs and program systems for the management of LAIR research data. These programs will (a) process the results of clinical and laboratory studies to derive their conclusions and to test applicability to specific military situations, (b) maintain and utilize effectively a repository of past research data for direct application to military problems and correlation with future approved military research, (c) evaluate data reported in the open literature to determine its applicability and to apply it to the military environment by correlation and transformation techniques, (d) support the requirements of privacy and the freedom of information acts, (e) provide data base and rapid analysis for information within the mission areas of LAIR in the event of mobilization.

24. (U) General purpose computer programs will be used to the greatest exten possible. Where the unique information processing requirements of a specific research protocol cannot be met by available general purpose computer programs, special purpose programs will be developed.

25. (U) 7810-7909. Special purpose programs were producted to support military biomedical research projects such as antimetabolite studies of irradiated foods, graphic recording on microfilm of reduced nutrition survey data, owl monkey behavior analysis, and petri plate counting studies. A generalized bar chart, histogram and linear plotting package has been designed and developed to fill the growing requirement for microfilm graphic output of publication quality. Network links among several of the presently independent computer systems are now under development. Several new statistical packages have been implemented for general use. A discrete-event simulation model was developed for assessing the impact of potential R&D improvements within the blood research area. Data acquisition software has been developed for stress and strain analysis for ligament repair studies. A calculator based data collection system was developed.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	055	Design of Support of Military Biomedical Research Information Systems

The following studies have been conducted under this work unit:

STUDY NO. 1	General mathematical and statistical systems development
STUDY NO. 2	Direct mathematical, statistical and data processing support of military biomedical research
STUDY NO. 3	Design of distributed information processing facilities
STUDY NO. 4	Data management systems development
STUDY NO. 5	Biomedical engineering and data acquisition

STUDY NOS. 1,2,3,4, and 5. As the use of quantitative techniques in biomedical research continues to expand, the need for better, more efficient, and more sophisticated data and information handling techniques has become more vital in the areas of data acquisition, mathematical modelling, graphics, data base management and statistical analysis. This work unit involves the design and development of tools and techniques for the acquisition and management of LAIR research data. These systems are necessary to (a) process the results of laboratory and clinical studies to derive their conclusions and to test their applicability to specific military situations, (b) maintain and utilize effectively a repository of past research data for direct application to military problems and correlation with future approved military research, (c) evaluate data reported in the open literature to determine its applicability and to apply it to the military environment by correlation and transformation techniques, (d) support the requirements of the privacy and freedom of information acts, (e) provide data base and rapid analysis of information within the mission area of LAIR in the event of mobilization.

BODY OF REPORT

WORK UNIT NO. 055 — Mathematical and Computer Support of Military Biomedical Research

STUDY NO. 1 — General mathematical and statistical systems development

PROBLEMS

Statistics is a collection of methods which allows one to make objective inferences about uncertain data. Data analysis, is a term used to describe computer techniques and methods of evaluating or handling data. The objectives of computer data analysis are (1) achieving a more specific description of what is loosely known or suspected; (2) finding unanticipated aspects of the data, and suggesting unthought-of-models for the data's summarization and exposure; (3) employing of data to assess the adequacy of a contemplated model; (4) attempting to provide incentives and guidance for further analysis of the data, and (5) keeping the investigator fully stimulated while he absorbs the feeling of the data and considers what to do next. The objective of this study is to provide the mathematical and statistical computer software necessary for comprehensive data analysis in support of the medical military research conducted at LAIR.

Although most data analysis procedures needed by LAIR investigators can be performed by using stand-alone prepackaged programs; such generalized programs may not readily lend themselves to implementation on minicomputers, such as the ECLIPSE C/330, because of size constraints. Therefore, large packaged programs are maintained at Lawrence Berkeley Laboratory (LBL).

RESULTS AND DISCUSSION OF RESULTS

LAIR maintains three statistical packages at Lawrence Berkeley Laboratory. The Generalized Research Analysis Statistical System (GRASS) is a package developed by the Information Sciences Group and designed to be easy to use for the researcher relatively unfamiliar with computers. GRASS has the capability to produce a variety of descriptive statistics, plots, histograms, and non-parametric tests. GRASS also has data transformation and data manipulation capabilities.

In addition to GRASS, a general statistical program (GENSTAT) developed at Rothamsted Experimental Station, England, is maintained by LAIR on the LBL CDC 7600. GENSTAT has a powerful statistical syntax which allows programs for complex statistical analyses to be written quickly and debugged. GENSTAT is particularly strong in the areas of generalized linear models and the analysis of designed experiments; it gives LAIR computer capabilities not otherwise available. GENSTAT also has many preprogrammed procedures including the ability to store and retrieve structured data sets.

The BMDP Biomedical Computer Programs developed at the UCLA Health Sciences Computing Facility are also maintained by LAIR at LBL. The BMDP package is a series of twenty-nine stand alone programs for statistical analysis. The programs share a free format English-based control language. BMDP has abilities in the area of data screening, analysis of qualitative data, analysis of variance and covariance, regression, multivariate analysis, and non-parametric statistics.

In addition to the packages maintained by LAIR, LBL supports the Statistical Package for the Social Sciences (SPSS), an integrated package for data manipulation and analysis. LBL also maintains a variety of FORTRAN subroutine libraries which may be used to build special purpose programs.

Limited statistical software is also available to researchers on the in-house ECLIPSE C/330 computer. MINITAB, a statistical computing system developed at Pennsylvania State University, provides data analytic capabilities to researchers who are analyzing small-to-medium sized data sets. MINITAB is easy to learn, well-documented, and runs interactively on the Eclipse computer. MINITAB capabilities include plotting, data manipulation and transformations, inferential and descriptive statistics, and matrix operations. This year work has begun on updating MINITAB with a new revision which enhances its many existing capabilities.

It is acknowledged that packaged programs will not solve all statistical computing problems at LAIR. Often special programs must be written to implement special techniques or data analytic methodologies. The Information Sciences Group believes that such programs are most easily developed on the ECLIPSE C/330. To facilitate building such programs, the International Mathematical and Statistical Library (IMSL) is maintained on the ECLIPSE C/330. IMSL contains approximately 200 mathematical and statistical FORTRAN subroutines. Several special purpose programs have been written with the use of IMSL building blocks. A new revision of IMSL has been obtained and is presently being installed.

CONCLUSIONS

A variety of statistical software are used to perform data analysis at LAIR. Program packages allow an investigator to select which statistical routines are most appropriate without having to master the computational techniques. LBL only supports one statistical package, i.e. the SPSS. A good remote computing site should provide several such packages, as well as a data base management system which is able to interface directly with statistical packages. The burden to maintain general purpose software should be on the remote computing facility, not on users such as LAIR.

RECOMMENDATIONS

The feasibility of obtaining remote batch processing services at another site which offers better supports for general statistical software should be investigated. Also we should investigate the installation and/or development of generalized statistical programs for the ECLIPSE.

STUDY NO. 2 — Direct mathematical, statistical and data processing support of military biomedical research

PROBLEM

Unique characteristics of modern military biomedical research require support to design and conduct experiments and to analyze experimental results. Technical support provided by this study is required to assure efficiency in data collection and to identify appropriate statistical analysis principles and techniques. Likewise, an objective of this study is to provide direct support to LAIR investigators and to aid data acquisition, management and analysis integral to experimental processes.

RESULTS AND DISCUSSION OF RESULTS

Consultation in experimental design, data analysis and in the use of mathematical and statistical computer software has been available to LAIR investigators as required. Objectives in formulating good experimental designs are (1) a formal definition of the primary goals of the experiment, (2) employment of a statistical model appropriate to the experimental material which provides unambiguous results, and (3) a design which is feasible within the working conditions of the investigator. After the data have been collected from the designed experiment, support is available to reduce and interpret the data statistically. Computing resources are available for all LAIR investigators in two data processing environments, the CDC 7600/6600 digital computers which are accessed remotely at the Lawrence Berkeley Laboratory (LBL) and the Data General ECLIPSE C/330 central minicomputer maintained at LAIR. Some data base management and statistical analysis functions which are not available on the C/330 require transferring data to the LBL environment for processing. The use of time sharing facilities at LBL for program development and on-line data analysis is nonexistent as the system is unresponsive, is less available to non-ERDA users and is less desirable considering the availability of time sharing facilities on the C/330.

Acquisition of data using instruments controlled by a desk top programmable HP9815S calculator has been successful. Communication of data from a calculator to the ECLIPSE C/330 for additional processing has been completed. Development of the system has been constrained, however, by the restricted size of the calculator memory space and required machine level programming.

The Information Sciences Group is one of the participating groups in the nutrition studies in support of the DoD Food Program. Computer programming, statistical, analysis of data, and data banking support provided by this group are described under Work Unit No. 086.

Division of Cutaneous Hazards

Analysis of dermatology clinic outpatient records collected over 3 years at Brooke, Fitzsimons, Letterman, and Walter Reed Army Medical Centers has been completed. Further processing of the data is not expected as the primary investigator and other key personnel associated with the study have separated from the Army. All software, data and documentation associated with the system have been copied and forwarded to USAHCSSA. The system has also been archived at LAIR.

The design of an ECLIPSE C/330 based system to manage mosquito repellent effectiveness testing data in a relational form was completed. Software has been designed and developed to unload the data base for transport to the LAIR ECLIPSE C/330 minicomputer. A program to report a repellent code-formulation dictionary has been designed and programmed and is currently being tested.

Division of Research Support

Animal Resources Group. Data processing support for investigators managing the owl monkey breeding colony at LAIR was discontinued because the colony was transferred to another Institute. Software developed and data acquired in this study have been archived at LAIR.

A system to support the collection of animal and food weights for antimetabolite studies of irradiation sterilized tests foods was developed. An HP9815S programmable calculator interfaced to an electronic digital balance is being used for weight collection. Programs to control weight collection, data unloading to the ECLIPSE C/330 minicomputer, and reporting of daily animal weight change and food intake have been implemented.

The HP9815S calculator based data acquisition system was also used to acquire animal weights in military toxicology studies. Likewise, the system has been programmed to calculate and store dosing data acquired in those studies. Communication of data from a calculator to the ECLIPSE C/330 for additional processing is featured. Memory space limitations and required machine level programming accounted for delays in developing functional software. Outstanding requirements exist for system software maintenance and development of software to report dosing data, to calculate diet preparation data, to manage collected data, and to unload data for more extensive statistical analyses.

Microbiological Services Group. On-going maintenance of the dictionary of standardized food class, food item, and organism designations is being supported. Retrieval and reporting of food class, food item, organism and bacteria count data collected in 1977 were completed.

Pathology Services Group. The HP9815S calculator based data acquisition system was programmed to control the collection of animal organ weights acquired in support of irradiated foods and toxicology studies. Communication of data to the ECLIPSE C/330 is supported. Manual input of organ weights is required, however, until a balance of milligram accuracy is interfaced to the calculator.

Radioisotope Services Group. On-going maintenance continues for programs which calculate beta counter calibration curve coefficients (program CRV) and disintegration rates (program DPM). We need to design programs to display and integrate peaks in disintegration rate counts and to calculate disintegration rates of spiked isotope solutions. We also need processing support to reduce and analyze radioimmunoassay data acquired from Institute gamma counters.

Nutrition Technology Division

Specialized programs were developed at LBL to generate publication quality microfilm recordings of bar charts and histograms summarizing nutrient intake data previously reduced from data collected at the Twentynine Palms Marine Corps Base and on the USS Saratoga-ship-afloat nutrition surveys. Data were recorded by using an SC4060 microfilm recorder available at the LBL facility.

Division of Surgery

Data analysis support was completed in the study of the effects of exercise on the strength of the anterior cruciate ligaments on rats. Statistical analyses were carried out, by using the Biomedical Computer Programs (BMDP). A program was developed at LBL with the aid of the GRAPHPAC package to present the statistical results as publication quality microfilm recordings of graphs.

Division of Blood Research

A program estimating optimal design points for nonlinear models was developed for the ECLIPSE to aid in the design of pharmacokinetic studies. Due to the limitations of the existing program, extensive research on the optimization techniques available, was carried out. It was concluded that the program should be revised for application on a broader spectrum of models. The revision of the program and research on the estimation of optimal design points are continuing.

Letterman Army Medical Center

Support in the design of experiments and the statistical analyses has been requested by various investigators. Several experimental designs and statistical analyses have been completed or are in advanced stages. Specifically, a design was completed on the experiment conducted to evaluate cardiovascular responses following administration of

anesthesia in patients with coronary artery disease. The data have been acquired and the statistical analysis is nearing completion.

CONCLUSIONS AND RECOMMENDATIONS

Appropriate use of statistical methods is essential to accomplish the scientific research mission at LAIR. Continued development of new techniques and implementation of accepted techniques for data analysis are essential to effective evaluation of research data. To reduce system development time and overall software maintenance requirements, the use of generalized software packages is preferred over reprogramming of existing functional software. The placement of data acquisition systems in several research support groups requires supporting data definitions that are established by investigators from outside the data acquisition environment. Processors for data acquisition functions which rely on supporting data must be configured with expanded memory, enhanced communications and direct access file and record retrieval mechanisms.

PUBLICATIONS

SPENCER, T.S., K.L. ZELLER, W.A. AKERS, AND W.H. LANGLEY: A Data Storage and Retieval System for a Mosquito Repellent Test Program. Report No. 67. San Francisco, California: Letterman Army Institute of Research, July 1979.

STUDY NO. 3 — Design of distributed information processing facilities

PROBLEM

Constantly changing information science technology and an increasing need for greater computing power in biomedical research require distributed computing systems which cannot be purchased as off-the-shelf items. The objective of this study is to develop this facility, specifically the hardware level of interfacing and the computer programs necessary to integrate many independent components into a coordinated facility.

RESULTS AND DISCUSSION OF RESULTS

Linkage between the in-house computing facility and Lawrence Berkeley Laboratory (LBL) has been established. The link has been tested and is fully functional. The link permits bidirectional transfer of files between the two facilities; then the complementary capabilities of both these facilities can be tapped. The data transfer process is fully protected for data integrity by error detection with automatic retransmission capability. From the LBL facility it is possible to transfer directly into the DoD Advanced Research Projects Agency Network (ARPANET).

Development of the LAIR Network (LAIRNET) has continued. The objective of this project has been to provide a point-to-point copy capability over asynchronous telephone lines between various laboratory computers within LAIR and the central Data General ECLIPSE C/330. The major aspect of this study, the supporting software, has been designed and written in FORTRAN. FORTRAN compilers exist for essentially all computers. The completed software is now being implemented and tested on a variety of machines within LAIR.

CONCLUSIONS AND RECOMMENDATIONS

More effective information processing systems have become essential. The work described above has resulted in the development of more effectively distributed information processing facilities for LAIR. Continued development is essential in the area of network protocols between dissimilar machines, data acquisition equipment, and microcomputer systems in order to make acquisition and processing of laboratory data more timely, more effecient, and more effective.

PUBLICATIONS

None

STUDY NO. 4 — Data management systems development

PROBLEM

The scientific researcher faces special problems in research data base management. Most generalized data base systems (such as System 2000) are not designed specifically with research data in mind. Although such systems will handle complex data structures adequately, they usually require high level programming ability and do not provide smooth interfacing with statistical analysis packages. Although some statistical packages have data management capabilities, they often lack adequate variable documenting capability, updating options, security protection, and the ability to define complex data structures conveniently. As the number of applications being developed on the C/330 increases, the need for data management features at several levels above existing programming language I/O processors is apparent. On the C/330 minicomputer a relational form of data base management is desirable. Features of a system would be that it is schema driven, supports loading, retrieval, updating and reporting of data and provides access control mechanisms. An outstanding feature of a relational data base is the ease in which related files may be interfaced to other systems.

RESULTS AND DISCUSSION OF RESULTS

Little progress has been reported by LBL staff regarding the implementation of the Scientific Information Retrieval (SIR) software package on the CDC 7600. SIR provides a data management capability which easily facilitates the input of nonsymmetrical data files in the Statistical Package for the Social Sciences (SPSS). Requirements for the package are diminishing.

No progress has been made in converting data bases managed under the Remote File Management System (RFMS) to System 2000. Requirements to convert the Mosquito Repellent Effectiveness Testing data base to System 2000 no longer exist because the data will be managed at LAIR. Analysis of data for the DoD Food Wholesomeness Study has been completed. Data are being unloaded and converted into a form transportable to other installations. Requirements for data management resources on LAIR's central minicomputer are presently being assembled. Emphasis is placed on features which support data, archival and uniform internal identification of file contents, and structure.

CONCLUSIONS AND RECOMMENDATIONS

If requirements for data processing support from the LBL computing facility should increase, evaluation of System 2000 would be in order. Likewise, management of hierarchical data bases for input to SPSS would require support of the SIR package. Due to lack of a data base management system on the C/330, many data base system functions are being reprogrammed in software to support an application. The availability of a system would significantly improve programmer production. Data identification features of a data management system are especially desirable as those features would directly provide information on data base contents and would standardize archival methods. Further inquiry into the availability of a relational data base management system for the ECLIPSE C/330 is recommended.

PUBLICATIONS

None

STUDY NO. 5 — Biomedical engineering and data acquisition

PROBLEM

Rising sophistication of biomedical research activities at LAIR has increased the requirements for biomedical engineering support. The objective of this study is to provide such support as needed by investigators at LAIR, to maintain responsive state-of-the-art techniques sufficient for future needs, and to collaborate with Institute investigators when appropriate.

RESULTS AND DISCUSSION OF RESULTS

Stress-Strain Data Acquisition of Ligament Repair Study. This project involves measuring the force maintained by ligaments and their resultant elongation as a function of time as they are mechanically pulled to rupture. Special purpose, automatic on-line data acquisition software has been created for this purpose and installed on the Data General NOVA 3/12. The data have been stored on disks in a form compatible with previously written off-line analysis programs.

Cardiopulmonary Analysis. This project involves on-line data acquisition of cardiopulmonary variables from anesthetized dogs, on-line analysis providing real-time feedback to the investigator in the form of analog graphic displays, and off-line analysis of the stored data. The cardiopulmonary effects of various kinds of anesthesia in dogs are described. Several portions have been completed.

On-line Outlier Detection. We have sought to devise a way to detect the occurrence of outlying (or obviously erroneous) data points or waveforms as they are being acquired on-line by computer without one's needing prior knowledge of the statistics or other characteristics of data. Mathematically, the problem is defined as deriving an algorithm which selectively rejects sequential data in a manner such that the mean of the accepted data converges faster than the mean of the total (accepted plus rejected) data, the variance of the accepted data is less than that of the total data and, under certain limiting assumptions, the error due to outliers in the mean calculated from the accepted data is smaller than the error in the mean of the total data. In statistical terms, such an algorithm produces quicker estimates of the mean of the sequentially arriving data.

Simulation of the problem has been archieved on the ECLIPSE C/330. Simulation runs have helped to identify the necessary trade-offs involved in on-line outlier detection and have suggested several algorithms which might prove successful. Testing of the algorithms is proceeding. Also, the mathematical framework necessary for defining the theoretical limits of successful application is being developed.

Base Excess Nomogram. A system of programs has been created and implemented on the Data General ECLIPSE C/330 which (1) accepts data concerning PCO_2 content, pH hemoglobin level, and measured base of excess of blood samples, (2) uses statistical curve fitting routines to calculate predicted values of pH as a function of base excess and PCO_2, (3) automatically modifies the fitted curves (and therefore the prediction of pH) to be consistent with both known and accepted hypothetical relationships between pH, PCO_2, and base excess, and (4) uses the resultant functions to produce computer plotted nomograms of PCO_2, pH hemoglobin, and base excess.

Data Acquisition in Support of Toxicology Studies. Initiation of studies in the toxicology of explosives and explosive by-products and requirements for compliance to the Good Laboratory Practice (GLP) regulations in the conduct of these studies necessitated a study to determine the availability of a data acquisition system which could be used to support GLP-type studies. Findings of the study indicated that the TOXSYS system developed and marketed by Beckman, Inc. was the only system of its type which provided protocol definition, data acquisition, and reporting applications extensively required for complete accountability. Hardware and software components of TOXSYS are being evaluated and the impact of its integration into the ECLIPSE C/330 environment is being determined. Enhancements to the C/330 required for support of TOXSYS have been identified; they include a COBOL compiler, the INFOS file management system, and an IBM-compatible floppy dual disk drive. While TOXSYS is not yet operable at LAIR, development of the HP9815S calculator based data acquisition system was continued and a functional description outlining plans for extending the functional capabilities of the system was prepared. The calculator based system is used to acquire animal body, foods and organ weight data.

CONCLUSIONS

Work Unit No. 055 will be terminated at the end of fiscal year 1980. The studies referred to in the above will continue to be addressed in the various other work units under the respective division.

RECOMMENDATIONS

Where applicable systems and programs require further development and perfection. A recent cost analysis reflects a substantial savings by augmentation to our current system. A machine having a larger work size, i.e. 32-bit, and virtual memory, would permit in-house support of the larger programs now supported by LBL. Plug and code compatability would be essential not only to assure networking as invisioned under the LAIRNET concept, but also to allow for transport and machine resource sharing between the C/330 minicomputer and the newly acquired machine.

PRESENTATIONS

1. HARRIS, D.A. Predicted trends in scientific computing due to new government regulations. International Symposium on Mini and Microcomputers, Montreal, Canada, 26-29 September 1979.

2. HARRIS, D.A., K.K. RIORDAN, M.I. TOWNSLEY, and R.B. WEISKOFF. Blood acid-base nomograms computer generated from raw data. Annual Conference on Engineering in Medicine and Biology. Denver, Colorado, 8-10 October 1979.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OB 6913	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM / A. WORK UNIT
78 10 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	066 APC 504R
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Physical, Chemical Characteristics of Human Stratum Corneum

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 07	79 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 79	1.0	19
CURRENT FISCAL YEAR 80	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Marshall, J.D., COL, MS

TELEPHONE: (415) 561-3600

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Division of Cutaneous Hazards

ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)

NAME: Reifenrath, William G., CPT, MSC

TELEPHONE: (415) 561-2421

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Eisenberg, George H.G., Jr., MAJ, MSC

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Stratum Corneum; (U) Absorption; (U) Permeability; (U) Water; (U) Water Vapor; (U) Chemicals; (U) Persistence; (U) Skin; (U) Human Volunteers

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective is to define the physical-chemical characteristics of the stratum corneum and its interaction with water, chemicals, ultraviolet radiation, and environment. These characteristics are fundamental to the etiology of several epidemic dermatological disorders caused by exposure of the soldier's skin to the environment and are also applicable to the behavior of topical preparations in human skin.

24. (U) Measurements of skin permeability and evaporation rates of insect repellents in vitro and in animals continue to provide guidelines for formulating compounds and vehicles to extend the duration on the skin and thereby increasing protection. These measurements also provide toxicological data for estimation of chemical exposure following topical application in man.

25. (U) 7810-7909. The effect of varying topical dose vs percent percutaneous penetration was studied for 5 mosquito repellents in the hairless dog. The evaporation/penetration characteristics of 5 mosquito repellents were determined in in-vitro permeability chambers. Agreement was found between calculated in vitro duration and mosquito repellent duration in man. The percutaneous penetration for flucinolone acetonide and diethylmalonate was determined in the hairless dog, the former for comparison to available data in man, the latter for comparison with diethylmalonate penetration of pig skin. This work unit is terminated due to a redirection of research activity. The research will be continued within a new comprehensive work unit.

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	066	Physical, Chemical Characteristics of Human Stratum Corneum

The hairless dog has been further investigated as an animal model for percutaneous penetration. Following the completion of studies conducted in FY 79, a total of 5 compounds has been tested in the hairless dog and man at a dose of 4 $\mu g/cm^2$, and the percutaneous penetration was found to correlate between man and dog ($r^2 = 0.92$).

The effect of chemical dose on percent percutaneous penetration was determined for 3 mosquito repellents (ethylhexanediol, m-deet and sulfonamide) using the hairless dog model. Mean percent penetration decreased with increasing dose for sulfonamide and m-deet and increased slightly for ethylhexanediol. However, these changes were not significant at the 95% confidence level.

An in vitro model has been used to study evaporation-penetration characteristics of several mosquito repellents (ethylhexanediol, m-deet, p-deet, sulfonamide and carbamide). In vitro durations, calculated from evaporation rates, have been correlated with in vivo duration ($r^2 = 0.94$) for four of the compounds (sulfonamide was found to have an in vitro duration in excess of the 12-hour test period and it had the longest in vivo duration of the compounds studied). Twelve-hour in vitro and hairless dog percutaneous penetration correlated ($r^2 = 0.96$) for four compounds (data for comparison of in vivo and in vitro percutaneous penetration of carbamide was not available).

BODY OF REPORT

WORK UNIT NO. 066 Physical, Chemical Characteristics of Human Stratum Corneum

PROBLEM

The skin of todays soldier is assaulted by a wide variety of potentially toxic chemicals, some of which are unique to his environment. Significant systemic exposure to these compounds may result via percutaneous penetration. It is therefore important to develop in vitro and animal test systems to estimate or predict human percutaneous penetration. In this study, the hairless dog was further investigated as an animal model for percutaneous penetration. In addition, an in vitro model for percutaneous penetration and evaporation loss measurements was evaluated using mosquito repellents.

RESULTS AND DISCUSSION OF RESULTS

Many studies of percutaneous penetration of topically applied substances have been conducted at a single chemical dose per unit skin area. This dose may or may not correspond to estimates of actual human exposure to the chemical. Percutaneous penetration of mosquito repellents, expressed as the percentage of applied dose, may be dose-dependent. In this study, the percent penetration of N,N-diethyl-m-toluamide (m-deet) and 2-ethyl-1,3-hexanediol (ethylhexanediol) was determined on hairless dogs at dosages of 4 and 320 ug/cm^2. In addition, the percent percutaneous penetration for n-butanehexamethyleneiminesulfonamide (sulfonamide, an experimental mosquito repellent) was determined at dosages of 100, 320 and 1000 ug/cm^2 on the hairless dog. Mean percent penetration decreased with increasing chemical dose for sulfonamide and m-deet, and increased slightly with ethylhexanediol; however, none of these changes were significantly different at the 95% confidence level.

An in vitro apparatus was used to study mosquito repellent evaporation and penetration characteristics with skin. The mosquito repellents m-deet, p-deet, ethylhexanediol, sulfonamide, and cyclohexamethylenecarbamide (carbamide) were studied. In vitro repellent duration, calculated from repellent evaporation rates from skin, was compared to in vivo duration at similar repellent dosage to assess the validity of the model. In vitro durations for m-deet, p-deet, ethylhexanediol, and carbamide correlated with in vivo duration (r^2=0.94), although in vitro duration was longer than in vivo duration. Sulfonamide, which lasted the longest in vivo, had an in vitro duration which exceeded the test period (12 hr). The 0-12 hour in vitro percutaneous

penetration correlated with the corresponding data from hairless dogs for the following compounds: m-deet, p-deet, ethylhexanediol, and sulfonamide (r^2=0.96). Hairless dog data were not available for carbamide. Percutaneous penetration was consistently higher with the in vitro model than with the hairless dog model.

The percutaneous penetration of flucinolone acetonide was determined in the hairless dog. This brings to 5 the number of chemicals studied in the hairless dog and man. Other compounds include m-deet, benzoic acid, progesterone, and testosterone. Percutaneous penetration in the hairless dog correlated with penetration determinations in man (r^2=0.92).

Percutaneous penetration of diethyl malonate, a nontoxic simulant for Soman, was determined in the hairless dog. Estimated percutaneous penetration was 4.1±1.8%, from a dose of 0.1 mg/cm^2 applied to the dogs' back. This value will be used for eventual comparison to diethyl malonate percutaneous penetration in other models.

CONCLUSIONS

The evaporation and/or penetration characteristics of a limited number of compounds have been studied in our in vitro system, in the hairless dog and in man. For several mosquito repellents, in vitro evaporation rates predict in vivo duration of effectiveness against mosquitoes.

For four compounds, in vitro penetration correlates with in vivo (hairless dog) penetration.

For the five compounds studied, percutaneous penetration in man and the hairless dog correlated.

RECOMMENDATIONS

Additional compounds, whose percutaneous penetration has been studied in man, should be tested for their percutaneous penetration in the in vitro system and in the hairless dog to validate further these models.

PUBLICATIONS

REIFENRATH, W.G., J.A. HILL, P.B. ROBINSON, D.L. McVEY, W.A. AKERS, M. ANGO and H.I. MAIBACH. Percutaneous absorption of C-14 labeled insect repellents in hairless dogs. J Environ Pathol Toxicol (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6783	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	067 APC 5041
b. CONTRIBUTING				
c. [redacted]	CARDS 1141			

11. TITLE (Precede with Security Classification Code)

(U) Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 - Biochemistry; 010100 - Microbiology; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 09	79 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	7.2	15
	CURRENT 80	0.0	11

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Marshall, J. D., COL, MS

TELEPHONE: (415) 561-3600

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Division of Cutaneous Hazards

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)

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TELEPHONE: (415) 561-5816

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS POC: DA

NAME: Kerbs, Sharon, Ph.D., DAC, Jaeger, J.,

NAME: DAC, Eisenberg, G.H.G., Jr.,MAJ,MS

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Skin; (U) Pathogenicity; (U) Iron; (U) Exocellular Products

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Under unfavorable conditions, epidermis of fungal infections can incapacitate 38 percent of combat soldiers within 16 days of first exposure, with the average period of incapacitation 7.3 days. The biochemical pathways fungi use to attack skin will be investigated. Mechanisms of iron acquisition by dermatophytes will be investigated. mechanisms of cell wall synthesis and production of toxic or irritant products by dermatophytes will be investigated.

24. (U) The approach will be 1) determine the iron requirements, the mechanisms of iron acquisition, the relation of iron acquisition capacity to pathogenicity of the dermatophytes, 2) determine conditions under which dermatophytes produce toxic or irritant products, 3) determine if the toxic or irritant products are significant factors in the pathogenesis of dermatophytosis.

25. (U) 7810-7909. Studies on the pathogenesis of fungi were continued and 18 different human dermatophytes were tested. Two media, Thiotone/egg yolk and Bactopeptone/egg yolk, were devised and tested. The new media stimulate growth with characteristics similar to those seen in natural fungal infections: in the absence of dextrose no microconidia are formed and only arthrospores and hyphae are produced. In vitro studies with *Candida albicans* indicate that the fungus grows in the mycelial form in the presence of less than 0.05mM ferric citrate and grows in the yeast form above 0.05mM ferric citrate. Experiments on folic acid deficient guinea pigs were terminated. The results indicate that fungal infections are more severe and disease lasts almost twoce as long in folic acid deficient animals. Testing of more effective antibacterial/antifungal agents for use by the soldier was begun. This work unit terminated due to redirection of research activity.

*Available to contractors upon originator's approval

* U.S. GPO: 1974—540-043/8691

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	067	Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections

The following investigation has been conducted under this work unit:

STUDY NO. 2 Morphological and chemostructural characterization of ontogenetic development of *Trichophyton mentagrophyte*

Under unfavorable conditions, epidemics of fungal infections can incapacitate 38 percent of combat soldiers within 16 days of first exposure, with the average period of incapacitation being 7.3 days. The biochemical pathways fungi use to attack skin were investigated. Mechanisms of iron acquisition by dermatophytes will be investigated. Mechanisms of cell wall synthesis and production of toxic or irritant products by dermatophytes were investigated. Studies on the pathogenesis of fungi were continued and 18 different human dermatophytes were tested. Two media, thiotone/egg yolk and bactopeptone/egg yolk, were devised and tested. The new media stimulate growth with characteristics similar to those in natural fungal infections, i.e., in the absence of dextrose no microconidia are formed and only arthospores and hyphae are produced. In vitro studies with *Candida albicans* indicate that the fungus grows in the mycelial form in the presence of less than 0.05 mM ferric citrate and grows in the yeast form above 0.05 mM ferric citrate. By harvesting large batches of mycelia, we were able to obtain a sufficient quantity of single arthroconidia to test them at three different concentrations on 30 guinea pigs and to compare these results to our established results with infections by the microconidium form of the same strains. Due to a change in mission of the division, reassignment of personnel and movement of laboratory equipment, the spores were stored in the refrigerator for several months before they could be used. Infection of guinea pigs with these microconidia did not correlate with our previous studies; no guinea pigs became infected. Only 4 out of 30 guinea pigs had visible lesions from quantitated arthrospores rubbed on their epilated skin.

BODY OF REPORT

WORK UNIT NO.	067	Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections
STUDY NO.	1	Morphological and chemostructural characterization of ontogenetic development of *Trichophyton mentagrophytes*

PROBLEM

Under unfavorable conditions, epidemics of fungal infections can incapacitate 38 percent of combat soldiers within 16 days of first exposure, with the average period of incapacitation of 7.3 days. In this study, the biochemical pathways fungi used to attack skin, the mechanisms of iron acquisition by dermatophytes, and the mechanisms of cell wall synthesis and production of toxic or irritant products by dermatophytes will be investigated. This study was continued from FY78 to determine if the arthroconidia produced in artificial medium are as infective as the microconidia. Guinea pigs served as test models. Arthroconidia are obtained from hypersegmented septae and, in some species, the conidium separates under conditions not yet known.

RESULTS AND DISCUSSION OF RESULTS

Studies on the pathogenesis of fungi were continued and 18 different human dermatophytes were tested. Two media, thiotone/egg yolk and bactopeptone/egg yolk, were devised and tested. The new media stimulated growth with characteristics similar to those seen in natural fungal infections, i.e., in the absence of dextrose no microconidia are formed and arthrospores and hyphae are produced. In vitro studies with *Candida albicans* indicate that the fungus grows in the mycelial form in the presence of less than 0.05 mM ferric citrate and grows in the yeast form above 0.05 mM ferric citrate. By harvesting large batches of mycelia, we were able to obtain a sufficient quantity of single arthroconidia to test them at three different concentrations on the skin of 30 guinea pigs and to compare these results to our established results with infections by the microconidium form of the same strains. Due to a change in mission of the division, reassignment of personnel and movement of laboratory equipment, the spores were stored in the refrigerator for several months before they could be used. Infection of guinea pigs with microconidia did not correlate with our previous studies; no guinea pigs became infected. Only 4 out of 30 guinea pigs had visible lesions from quantitated arthrospores rubbed on their epilated skin.

CONCLUSIONS

None

RECOMMENDATIONS

The studies with arthrospores should be repeated.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6302	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 15	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. [redacted]	62772A	3S162772A814	00	020 APC 505H
b. [redacted]	61102A	3M161102BS02	00	071
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) The Response of Muscle to Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	1.0	34
	CURRENT 80	3	92

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Scott, Rhonda L., CPT, MSC

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Skeletal Muscle; (U) Myoglobin; (U) Metmyoglobin Reductase; (U) Heatstroke; (U) Muscle Injury; (U) Oxygen Utilization by Muscle

23 TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The acutely injured soldier develops negative nitrogen balance and loses muscle mass through mechanisms which are unknown. One of the factors which may be involved is myoglobin, a heme-protein which transports oxygen within muscle cells. Myoglobin and its overall metabolic relationships within the muscle cell serve as useful markers in the study of muscle injury. Injured muscle loses myoglobin into the peripheral circulation where it may cause secondary renal damage for unknown reasons. Failure of myoglobin to maintain sufficient intracellular oxygen supply may lead to decreased energy production, weakness, and failure of mechanisms upon which recovery from injury depend.
24. (U) Selected aspects of the effects of injury on muscle will be evaluated. Strategies designed to minimize and/or reverse the detrimental effects of injury on muscle will be determined. The effects of muscle injury on other body systems, including the kidney, will be studied. The relationship between myoglobin (and its associated reactions in the muscle cell) and immobilization-induced muscle atrophy, exercised-induced muscle hypertrophy, and recovery from injury will be studied.
25. (U) 79 08 - 79 09 Detailed studies concerning metmyoglobin reductase have been reported (J. Biol. Chem. 254: 6505, 1979). The influence of diet and exercise on myoglobin and metmyoglobin reductase were evaluated in the rat. The activity of metmyoglobin reductase was increased in the group undergoing the highest level of activity and decreased in the groups whose diets were restricted. These changes were seen only in the soleus muscle; the heart, psoas, and quadriceps were unaffected. Exercise increased myoglobin only in the quadriceps and soleus muscles. These data illustrate the differing adaptive patterns of myoglobin and metmyoglobin reductase.

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M161102BS02 Basic Mechanisms of Recovery from Injury

WORK UNIT NO. 071 The Metabolic Response of Muscle to Injury, Exercise, and Diet in Health and Disease

The following investigations have been conducted under this work unit:

STUDY NO. 1 Studies concerning the mechanism which controls the redox state of myoglobin

STUDY NO. 2 Effect of wounding on muscle metabolism

STUDY NO. 1. Beef heart muscle contains an enzyme which will rapidly and directly reduce metmyoglobin in vitro. Reduction rates of metmyoglobin are far greater than any previously reported for nonspecific or nonenzymatic systems. The enzyme is NADH-dependent and requires the presence of ferrocyanide ion for in vitro assay. The artificial electron carriers, dichlorophenolindophenol and methylene blue, are not required. Nonenzymatic reduction of metmyoglobin, which has previously been reported, was not encountered under the assay conditions. The methods for preparation of beef heart myoglobin substrate and for purification of the enzyme have been established.

Studies were carried out to evaluate selected aspects of heme protein metabolism in the rat. Two levels of treadmill exercise and three levels of dietary restriction were imposed on growing male rats over a 12-week period. The activity of NADH-metmyoglobin reductase was increased in the group undergoing the highest level of training and decreased in the groups whose diet was restricted by 25% and 35%, respectively. These changes were seen only in the soleus muscle. Other muscles, including the heart, psoas, and quadriceps were unaffected by either exercise or diet. Both levels of exercise were effective in increasing muscle myoglobin concentration, but only in the quadriceps and soleus muscles. These data illustrate the adaptive nature of muscle myoglobin and NADH-metmyoglobin reductase. They also illustrate the different adaptive patterns of these two components of muscle.

STUDY NO. 2. A potential mechanism to preserve tissue composition and reduce muscle catabolism following wounding is the postinjury adminstration of proteolytic inhibitors. Laevadosin is a drug produced by Boehringer-Mannheim and used in Europe as an agent in the treatment of muscular dystrophy. This drug was administered to wounded and normal rats and its effect on selected metabolic parameters was

measured. Daily intraperitoneal administration had no effect on weight gain or efficiency of feed utilization over a 17-day experimental period. Tissue levels of glutamic oxalacetic transaminase and creatine phosphokinase were depressed following wounding, but were not affected significantly by Laevadosin administration. Free cathepsin D activity in nontraumatized tissue was similarly unresponsive; however, in wounded muscle this enzyme was increased significantly by Laevadosin treatment. The possible significance of this finding in relation to wound healing and pharmacological manipulation to reduce postinjury catabolic response will require further exploration.

BODY OF REPORT

WORK UNIT NO.	071	The Metabolic Response of Muscle to Injury, Exercise, and Diet in Health and Disease
STUDY NO.	1	Studies concerning the mechanism which controls the redox state of myoglobin

PROBLEM

Muscle function is impaired in soldiers either directly by injury or indirectly by immobilization. In order to facilitate healing and to reverse atrophy of muscle, it is necessary to understand the mechanisms involved in exercise-induced hypertrophy and immobilization-induced atrophy of muscle. Muscle is the only tissue which contains myoglobin, the presence of which subserves functions whose precise nature remains uncertain. Since myoglobin is a heme protein, it is presumed that its function, in part, is related to oxygen transport/storage in the muscle cell. It is postulated that myoglobin may be centrally involved in the energy dependent processes of muscle via this function as an intracellular carrier of oxygen.

Myoglobin, like hemoglobin, undergoes freely reversible oxygenation in order to carry out its oxygen transport function. Myoglobin is nearly 20 times more easily oxidized than hemoglobin. The oxidized forms of hemoglobin and myoglobin (methemoglobin and metmyoglobin, respectively) are incapable of carrying oxygen. The red blood cell possesses several enzymatic mechanisms which maintain hemoglobin in the functional reduced state. We have isolated, purified, and characterized an enzyme (NADH-metmyoglobin reductase) which actively reduces metmyoglobin in vitro. The influences of exercise and dietary restriction on muscle myoglobin and metmyoglobin reductase were studied in the rat.

RESULTS AND DISCUSSION OF RESULTS

Metmyoglobin reductase has been repeatedly purified maximally to as much as 2000-fold, yielding specific activities in excess of 150,000 U/mg protein. The properties and characteristics of the highly purified enzyme and the in vitro assay system have been established. Activity was dependent on a properly prepared substrate, NADH, and $K_4Fe(CN)_6$. The enzyme has a pH optimum about 6.5, a Km of 5.0×10^{-5}M, and is unaffected by the absence of O_2. Molecular weight estimation by gel permeation chromatography and sodium dodecyl sulfate-gel electrophoresis confirmed a molecular weight around 30,000. Purified enzyme did not react with lipoamide which clearly differentiated it from diaphorase.

Studies with inhibitors demonstrated sensitivity to sulfhydryl inhibitors and to flavin antagonists. The addition of flavin mononucleotide stimulated in vitro activity. The temperature optimum was 37 C; heating to 50 C abolished activity. The addition of bovine serum albumin or myoglobin to the highly purified enzyme protected the activity when stored at -20 C. Bovine erythrocyte methemoglobin reductase was purified by similar techniques and compared to the purified muscle enzyme. These studies demonstrated that the reductases from muscle and erythrocytes were similar. On acrylamide gel electrophoresis, however, the two enzymes produced different activity patterns which speaks against their being the same.

Male rats weighing approximately 130 g were divided into 6 treatment groups. Groups 1 and 2 were trained by treadmill running at high (Group 1) and moderate (Group 2) levels of exercise, while Group 3 served as a pair-fed sedentary control. Groups 4 through 6 remained sedentary in cages and received varying quantities of diet. At the end of the 12-week period of training and diet the rats were killed. The heart, psoas, quadriceps, femoris, and soleus muscles were removed and frozen at -20 C until assayed. For Groups 1, 2, and 3, the experimental design ensured similar dietary intakes. The weights of Groups 1 and 2 were similar, but the weight of the pair-fed sedentary control group (Group 3) was higher, illustrating the importance of physical activity in the utilization of calories. The dietary manipulations in Groups 4, 5, and 6 resulted in highly statistically significant differences in mean weights. Overall growth proceeded normally, albeit at different rates. The training regimen had no effect on the activity of methemoglobin reductase. The mean hemoglobin level in the high exercise group (Group 1) was significantly lower ($p \leq 0.02$) than in the other groups (15.6 ± 0.2 vs. 16.2 ± 0.2, respectively). In Groups 1, 2, and 3, in the heart, psoas, and quadriceps, the mean metmyoglobin reductase activities differed from each other, but failed to change in response to exercise. In Group 1, the activity of metmyoglobin reductase in the soleus muscle was significantly higher ($p \leq 0.01$) than in the other groups. In Groups 4, 5, and 6, alterations in diet had no effect on the mean activity of metmyoglobin reductase in either the heart, psoas, or quadriceps. Enzyme activity in the soleus, however, was responsive to diet, showing progressive decrease in response to diminished dietary intake. Exercise had no effect on muscle myoglobin concentration in either the heart or psoas muscles. The quadriceps and soleus muscles both demonstrated increased muscle myoglobin in the exercised animals. At both levels of exercise in both muscles, myoglobin concentration was significantly higher than in the sedentary pair-fed control animals. In Groups 4, 5, and 6, which received varying levels of dietary intake, there were no differences in muscle myoglobin concentration.

Other studies related to this area of investigation were carried out in collaboration with MAJ E. Wayne Askew, under Work Unit No. 059, Biochemical Adaptations and Dietary Interactions of Exercise Training. Those studies are reported separately.

CONCLUSIONS

In our studies, we have identified and characterized metmyoglobin reductase from bovine heart muscle. This enzyme is a distinct entity in muscle which is not diaphorase or contaminating methemoglobin reductase. Its properties are in keeping with the conditions of pH and temperature which are known to exist in exercising muscle. The discovery of this enzyme and its characterization add a new dimension to the consideration of the role of myoglobin in muscle function.

The studies have shown that each muscle or muscle group has its own characteristic level of both myoglobin and metmyoglobin reductase. Exercise is a well-known stimulus to muscle myoglobin production, but only in those muscles which are being stressed by the training regimen. The increase in muscle myoglobin is not a nonspecific or generalized response to exercise since it is not seen in all muscles, but only those which are carrying out the exercise. Myoglobin levels in muscle are not influenced appreciably by diet. The activity of metmyoglobin reductase increased significantly in only the soleus muscles from the animals in Group 1. The response appeared to be related to the level of activity. It is clear that the adaptive response of metmyoglobin reductase to exercise, like that of myoglobin, is neither generalized nor nonspecific since it occurred only in the soleus muscle. The enzyme activity decreased only in the soleus muscle of the groups undergoing dietary restriction. These data indicate that differing mechanisms mediate the adaptive responses of myoglobin and metmyoglobin reductase which are seen in response to training regimens and to diet. The reasons for the particular sensitivity of the soleus muscle are unknown. Of particular interest was the decreased hemoglobin levels in the high exercise group. Further studies related to this phenomenon were carried out under Work Unit No. 059, and are reported separately.

RECOMMENDATIONS

These studies should be continued. The mechanisms which underly the decreased hemoglobin levels in endurance training should be sought. The use of myoglobin and metmyoglobin reductase as markers of muscle injury and as a means of quantitation should be continued.

PUBLICATIONS

1. HAGLER, L., R.I. COPPES, Jr., and R.H. HERMAN. Metmyoglobin reductase: Identification and purification of a reduced nicotinamide adenine dinucleotide dependent enzyme from bovine heart which reduces metmyoglobin. J Biol Chem 254: 6505-6514, 1979

2. HAGLER, L., R.I. COPPES, Jr., E.W. ASKEW, A.L. HECKER, and R.H. HERMAN. The influence of exercise and diet on myoglobin and metmyoglobin reductase in the rat. J Lab Clin Med 95: 222-230, 1980

STUDY NO. 2 Effect of wounding on muscle metabolism

PROBLEM

Minimizing postinjury mobilization of functional muscle mass represents a significant problem in the treatment of trauma patients. Nutritional, hormonal, and pharmacological treatments designed to promote healing and preserve normal body composition need to be developed. Muscular dystrophy represents a type of wasting which is similar in some respects to the loss of muscle mass seen during starvation and/or during trauma recovery. Thus, one approach to identify agents for the treatment of the post-trauma catabolic state is to examine the metabolic effects of drugs already in use for the treatment of muscular dystrophy. Laevadosin, a product composed of ATP, ADP, AMP, GMP, adenosine, guanine, and uridine was selected for this initial investigation.

RESULTS AND DISCUSSION OF RESULTS

Rats were injected intraperitoneally with either 0.2 ml Laevadosin per 100 g body weight, or saline for 10 days before wounding. Wounding was accomplished by injecting 5.0 cc of a 0.5% solution of λ-carrageenan into the muscle of one hind limb. Seven days following wounding, the animals were anesthetized and leg muscle tissue removed and analyzed for cathepsin D, GOT, GPT, and CPK activity. GOT and CPK activities were depressed following wounding, but there were no differences between the Laevadosin treated or untreated animals. CPK was also depressed following wounding, but activity was increased significantly due to Laevadosin in both control and wounded tissue. Cathepsin D was increased 6-fold by wounding. Whereas Laevadosin had no effect on nonwounded tissue, it caused a further increase in wounded muscle cathepsin above the saline injected control. As cathepsin D is believed to be partially responsible for protein turnover, it is possible that part of the beneficial effect of Laevadosin in muscular dystrophy could be its effect on cathepsin activity.

These data indicate that there is an increase in some selected enzymes of protein and amino acid metabolism. There were no deleterious effects observed on nonwounded animals. A time-course study of wound healing using a suitable wound model could be expected to make obvious any beneficial effect of Laevadosin.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 0B 6800	2. DATE OF SUMMARY* 79 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 78 10 01	4. KIND OF SUMMARY H. TERMINATION	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3M162772A810		00	003 APC 504R		
b. CONTRIBUTING							
[redacted]	CARDS 114f						

11. TITLE (Precede with Security Classification Code)*
(U) More Effective Topical Repellents Against Malaria - Bearing Mosquitoes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 11	79 10	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: Not Applicable
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 79	2.8	98
	CURRENT 80	0.0	00

19. RESPONSIBLE DOC ORGANIZATION
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NAME: Eisenberg, George H.G.,Jr.,MAJ,MSC

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Tropical Diseases; (U) Topical Repellents; (U) Human Volunteers; (U) Insect Repellent; (U) Mosquito; (U) Skin; (U) Stratum Corneum; (U) Polymer formulations.

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Punish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objectives are to discover a long-lasting water and abrasion resistant topical repellent formulation that will protect soldiers against malaria-bearing mosquitoes and other vectors of militarily important diseases; to develop in vitro test methods to determine physical properties of repellent formulations which are tested in the in vivo screening program, and by correlating the in vitro and in vivo test results to predict evaporation, penetration, and repellent-skin interactions which will aid in designing new repellents.

24. (U) Physical properties and efficacy of repellents and formulations will be determined by partition coefficient, relative solubility, volatility, surface activity, and duration on animals and man. With the use of a computer base, for statistical analyses of repellent test results, individual characteristics which can be enhanced to promote longer duration of repellent protection will be determined.

25. (U) 78 10 - 79 09. Surface tension determinations for seven known repellent chemicals were completed. Contact angle, film hardness and drying time determinations were completed on approximately 30 repellent formulations. A program to update entries in the human repellent data management system was completed. In accordance with EPA regulations, toxicological testing of a new candidate mosquito repellent (N-octyl-glutarimide) was begun. After determination of its purity, the LD50 for N-octyl-glutarimide in male and female Sprague Dawley rats was established at 4000 and 6300 mg/kg respectively. At necropsy, male and female animals that died of natural death showed no organ abnormalities. Terminated for consolidation with similar work in a new work unit.

*Available to contractors upon originator's approval

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M162772A810 Military Skin Disease

WORK UNIT NO. 003 More Effective Topical Repellents against Malaria-bearing Mosquitoes

The following investigations have been conducted under this work unit:

STUDY NO. 1 Information retrieval system for insect repellents data

STUDY NO. 2 Formulation and in vitro evaluation of mosquito repellents

STUDY NO. 3 Testing of non-standard insect repellents and repellent formulation in volunteers

STUDY NO. 1. In collaboration with the Information Sciences Group, LAIR, a computer program was devised to update entries for a relational computer data base of mosquito repellent effectiveness, test conditions, volunteer characteristics, and repellent physical properties. Measurement of surface tension for several mosquito repellents was completed for entry into the data base.

STUDY NO. 2. During FY78, two new mosquito repellents were selected for advanced testing. During FY79, an LD_{50} determination in male and female rats was completed for one of the compounds.

STUDY NO. 3. N,N-Diethyl-m-toluamide (m-deet) was formulated with several acrylate polymers in ethanol solution and various silicone polymers in isopropanol suspension, varying the ratio of polymer to m-deet. Formulations which had short drying times were evaluated for film hardness and elasticity, and contact angle with water. For the acrylate formulations, decreasing of the ratio of polymer to m-deet increased drying time, decreased film hardness and elasticity, and decreased the contact angle with water. Silicone formulations generally did not dry to a film within eight hours and had low contact angles with water.

BODY OF REPORT

WORK UNIT NO.	003	More Effective Topical Repellents Against Disease-Bearing Mosquitoes
STUDY NO.	1	Information retrieval system for insect repellents data

PROBLEM

The Division of Cutaneous Hazards currently has on computer records the results of approximately 250 separate repellent tests involving 277 different volunteers, in which 130 different repellent chemicals or formulations have been tested at LAIR. In addition, information is on file regarding test conditions, individual volunteer characteristics, and physical-chemical characteristics of the repellent chemicals. During FY79, the surface tension of several standard repellent compounds was determined and coded for entry into the data base. These data are, in part, managed by the Remote File Management System which has the following disadvantages: no data editing during data entry, no sort capability, no real time sharing and interactive processing, and limited statistical options. No provision exists in this system for animal test data, although 90 separate tests with 50 different repellents or formulations are in a data file for our hairless dog testing. Efficient computerized access and statistical analysis of these data are essential for guiding future studies in repellent testing and research.

RESULTS AND DISCUSSION OF RESULTS

During FY79, efforts focused on the development of the relational computer data base for volunteer repellent test results, test conditions, volunteer characteristics, and repellent characteristics. During this time, a program was developed to update entries in the data base. Work was halted on the development of a computer data base for animal test data.

CONCLUSIONS

Data relating to the efficacy of chemicals as mosquito repellents, the physical-chemical properties of the chemicals, the characteristics of volunteers who participated in the tests, and the test conditions have been accumulated and updated for a relational data base.

RECOMMENDATIONS

Update of the data associated with the volunteer repellent data management system should continue. Future test data and physical-chemical properties of repellents should be added to the system as they become available.

More Effective Topical Repellents Against Disease-Bearing Mosquitoes

PUBLICATIONS

SPENCER, T.S., K.L. ZELLER, W.A. AKERS, and W.H. LANGLEY. Data Storage and Retrieval System for a Mosquito Repellent Test Program. Interim Report, 1977, Institute Report No. 67, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129, 1979

STUDY NO. 2 Formulation and in vitro evaluation of mosquito repellents

PROBLEM

Our military has a vital interest in the development of improved mosquito repellents, since military operations may take place in areas where mosquito-borne diseases are endemic. In addition to having improved efficacy, a better repellent must also be safe for human use. In this work unit, a progressive toxicology testing procedure has been developed. Two new repellents are currently undergoing Phase I toxicology tests.

RESULTS AND DISCUSSION OF RESULTS

During FY79, two compounds, 165-67 and 835-23A, were selected for advanced testing. Larger quantities of both compounds were synthesized and checked for purity. In accordance with Environmental Protection Agency regulations, the LD_{50} of compound 835-23A in male and female Sprague Dawley rats was established at 4000 and 6300 mg/kg respectively. At necropsy, male and female animals that died of natural death showed no organ abnormalities.

CONCLUSION

The results of the above LD_{50} tests for 835-23A would not preclude the compound from being advanced for further toxicology tests.

RECOMMENDATION

Phase I toxicology for compounds 835-23A and 165-67 should be completed to allow a decision to be made on testing the compounds on man.

PUBLICATIONS

HILL, J.A., P.B. ROBINSON, D.L. McVEY, W.A. AKERS and W.G. REIFENRATH. Evaluation of mosquito repellents on the hairless dog. Mosquito News, 39:307-310, 1979

More Effective Topical Repellents Against Disease-Bearing Mosquitoes

STUDY NO. 3 Testing of non-standard insect repellents and repellent formulation in volunteers

PROBLEM

Mosquitoes as vectors transmit malaria, dengue, chickugunya, viral encephalitis, and many other diseases. These diseases, endemic in many parts of the world, cause over two million deaths each year. Since our military has a global responsibility, exposure to mosquitoes is current and a reality. The performance of the current military repellent (m-deet, N,N-diethyl-m-toluamide) is limited by excessive evaporation from the skin surface, abrasion, water wash-off and penetration into the skin. To improve the performance of m-deet, the repellent was formulated to reduce its loss from the skin surface. Since the evaluation of these formulations on volunteers is expensive and time-consuming and requires toxicological testing as a prerequisite, the development of in vitro methods to screen formulations for various properties is required.

RESULTS AND DISCUSSION OF RESULTS

N,N-Diethyl-m-toluamide (m-deet) was formulated with several acrylate polymers in ethanol solution and various silicone polymers (varying the ratio of polymer to m-deet).

Films of formulations were cast with a Gardner knife and examined for the following properties:

Drying time. Films were cast on glass microscope slides and the time it took for the cast film to become non-adherent to cotton was determined. Films with long drying times are not cosmetically acceptable.

Sward hardness. Films were cast on aluminum plates for those formulations which had short drying times. Film thickness and Sward hardness were then determined. The modulus of elasticity of the film was then calculated. A high modulus of elasticity may mean that the film is less flexible than the skin, in which case it will crack and peel from the skin.

Contact angle with water. Films were cast on glass plates and the contact angle that droplets of water make at the film surface was determined. High contact angles of water to the films indicate that the surface is difficult to wet and that the formulation has resistance to water wash off.

Increasing the ratio of acrylate polymer Carboset 526, 525 or 514, to m-deet resulted in decreasing drying time, increasing contact angle

with water, and increasing modulus of elasticity. The lower ratio formulations provide wash resistance, but remain sticky or tacky for too long a time. The higher ratio formulations approach reasonable drying time, but are too inflexible and some were observed to crack and peel from the skin surface of hairless dog skin. Carobset 515, a low molecular weight acrylate polymer, either by itself or in combination with m-deet, provided long drying times and was a poor film former. Formulations of m-deet and various silicone polymers produced films with long drying times and low contact angles with water. In volunteer testing, silicone formulations with m-deet provided poor protection against water wash off.

CONCLUSIONS

Changes in the physical properties of film forming polymers are reflected in their in vivo cosmetic properties.

RECOMMENDATIONS

Measurement of contact angle, film harness, and drying time of films cast by mosquito repellent formulations, together with the performance of the formulations against mosquitoes should be used to select the best candidates for advanced testing.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DA OB 6915	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. DOWN SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A810	00	006 APC 504T
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)*

(U) Skin Diseases Among Soldiers

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
68 01	79 10	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: Not Applicable d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 79	1.1	19
CURRENT FISCAL YEAR 80	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: Eisenberg, George H.G.,Jr.,MAJ,MSC

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Human Volunteers; (U) Occupation; (U) Diagnosis; (U) Skin; (U) Survey; (U) Soldiers; (U) Morbidity

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objectives are to determine the type and frequency of potentially disabling skin diseases among soldiers in various environments, to conduct trials of potential preventive and therapeutic agents against the common disabling dermatoses that afflict military personnel, and to develop or improve methods of studying militarily relevant skin diseases under field conditions.

24. (U) Conduct a 3-year study of all outpatient visit diagnoses at the 4 Army dermatological centers; determine prevelence of contact sensitivities to materials that may produce contact dermatitis in soldiers; develop and test performance characteristics of new techniques for diagnosing skin diseases in the field.

25. (U) 78 10 - 79 09. A program in dermatotoxicology with subdisciplines in skin irritation and skin sensitization was begun. Experiments on folic acid deficient guinea pigs were terminated. The results indicate that fungal infections are more severe and the disease lasts almost twice as long in folic acid deficient animals. This work unit is terminated due to a redirection of research activity and consolidation of toxicological investigations under another work unit.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M162772A810 Military Skin Disease Skin

WORK UNIT NO. 006 Skin Diseases Among Soldiers

The objectives of this work unit were to determine the type and frequency of potentially disabling skin diseases among soldiers in various environments, to conduct trials of potential preventive and therapeutic agents against the common disabling dermatoses that afflict military personnel, and to develop or improve methods of studying militarily relevant skin diseases under field conditions. A 3-year study of all outpatient visit diagnoses at 4 Army dermatological centers was conducted to: (a) determine the incidence of contact sensitivities to materials that may produce contact dermatitis in soldiers and (b) assess the performance characteristics of new techniques for diagnosing skin diseases in the field. A program in dermatotoxicology with subdisciplines in skin irritation and skin sensitization was begun. Due to mission realignment, toxicology testing was transferred to another division. With transfer of total mission for conducting clinical research to Health Services Command, planning for new clinical studies was discontinued and activities in this work unit were halted when the contact dermatitis survey was completed.

BODY OF REPORT

WORK UNIT NO. 006 Skin Disease Among Soldiers

PROBLEM

Ninety-five percent of dermatological patients are treated as outpatients, which is the American tradition of trying not to hospitalize patients with skin diseases. The Army efficiently collects elaborate data on diagnosis, treatment, and disposition of hospitalized patients, but little demographic data are saved on outpatients. Little data are available in the literature on the skin diseases of the soldiers, on the incidence, morbidity, disposition, and frequency of dermatological diagnoses in the military dermatology clinics. A computer supported dermatological outpatient data system was implemented to provide the physician and the administrator with information concerning the outpatient load, diagnostic and therapeutic problems encountered, need for varying types of supporting personnel, medical equipment and drug requirements, and disposition of patients. The 54 most frequent diagnoses have been stratified by diagnosis, hospital, age groups, ethnic origin, sex, initial visits, and military status (active duty, retired, and dependent).

RESULTS AND DISCUSSION OF RESULTS

Due to mission realignment, toxicology testing was transferred to another division. With transfer of total mission for conducting clinical research to Health Service Command, the results of the contact dermatology study (see Annual Report, FY 78) were submitted for publication and no new studies were initiated.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

PRYSTOWSKY, S.D., A.M. ALLEN, R.W. SMITH, J.H. NONOMURA, R.B. ODOM, M.J. BUDNIK, and W.A. AKERS. Allergic contact hypersensitivity to nickel, neomycin, ethylenediamine and benzocaine: Relationships between age, sex, history of exposure, and reactivity to standard patch tests and use tests in a general population. Arch Dermatol 115:959-962, 1979

PRYSTOWSKY, S.D., J.H. NONOMURA, R.W. SMITH, and A.M. ALLEN. Allergic hypersensitivity to neomycin: Relationships between patch test reactions and "use tests." Arch Dermatol 115:713-715, 1979

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6119	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A810	00	015 APC 504A
b. CONTRIBUTING				
XXXXXXXX	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Development of Improved Insect Repellents for Military Use

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

005900 Environmental Biology; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 - 10	79 - 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: Not Applicable d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	8.1	133
FISCAL YEAR CURRENT 80	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Rutledge, L.C., DAC

NAME: Wirtz, R. A., CPT, MSC POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Repellent; (U) Insect Repellent; (U) Mosquito Repellent; (U) Diethyl Toluamide

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop safe, long-lasting, cosmetically acceptable topical preparations for protection of soldiers against disease-carrying and pest species of insects to increase the effectiveness of units in combat and other operational situations.

24. (U) Selected insects of military medical importance will be colonized for repellent testing; improved repellent testing procedures will be developed; new formulations of diethyl toluamide and ethyl hexanediol will be evaluated for Army use; various compounds will be evaluated for adoption as general use and special purpose repellents.

25. (U) 78 10 - 79 09. Colonies of representative mosquitoes, sand flies, bugs and ticks were continued, and a colony of the oriental rat flea was established. Repellent tests against the oriental rat flea and a softbacked tick (*Ornithodoros parkeri*) were initiated. ED50 and 4-hour ED50 test methods for evaluating mosquito repellents on laboratory mice, dogs and human volunteers were developed, and standard methods for evaluating mosquito repellents on human volunteers were submitted to the American Society for Testing and Materials (ASTM). Advanced testing of compounds provided by Stanford Research Institute and the Department of Agriculture (FY 1976 to FY 1978) was continued. Two compounds, SRI 835-23A (N-(N-octyl)-glutarimide) and SRI 165-67 (N-(N-hexyl)-2-oxazolidine), were referred to the acute-phase toxicology stage of development. Testing of 26 new polymer formulations using diethyl toluamide, ethyl hexanediol and cyclohexamethylenebutane sulfonamide as active agents was initiated. Questionnaires were circulated at Army posts to document the usage of existing Army and commercial repellents and to aid in establishment of requirements for new repellent systems. Terminated for consolidation with similar work in a new work unit.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M162772A810 Military Skin Diseases

WORK Unit NO. 015 Development of Improved Insect Repellents for Military Use

The following investigations have been conducted under this work unit:

STUDY NO. 1 Colonization of selected insect vectors of disease

STUDY NO. 2 Entomological evaluation of insect repellents

STUDY NO. 3 Development of improved tick repellents for military use

STUDY NO. 4 Development of duration testing methods for mosquito repellents

Colonies of representative mosquitoes, sand flies, bugs and ticks were maintained, and colonies of the oriental rat flea and *Triatoma barberi* were established. Repellent tests against the oriental rat flea and a soft-backed tick (*ornithodoros parkeri*) were initiated. ED_{50} and a 4-hour ED_{50} test methods for evaluating mosquito repellents on laboratory mice, dogs, and human volunteers were developed and standard methods for evaluating mosquito repellents on human volunteers were submitted to the American Society for Testing and Materials (ASTM). Advanced testing of compounds provided by the Stanford Research Institute and the Department of Agriculture (FY 1976 to FY 1978) was continued. Two compounds, SRI 835-23A (N-(n-octyl)-glutarimide) and SRI 165-67 (N-(n-hexyl)-2-oxazolidine), were referred to the acute-phase toxicology stage of development. Testing of 28 new polymer formulations of diethyl toluamide, ethyl hexanediol, and cyclohexamethylene butane sulfonamide was initiated. Questionnaires were circulated at 7 Army posts to document the usage of existing Army and commercial repellents and to establish requirements for new repellent systems.

BODY OF REPORT

WORK UNIT NO.	015	Development of Improved Insect Repellents for Military Use
STUDY NO.	1	Colonization of selected insect vectors of disease

PROBLEM

Laboratory studies concerning the development of improved insect repellents depend heavily on the presence of a constant supply of representative insect species. Laboratory colonies provide an economical, close at hand resource for repellent study in place of travel to distant areas where insects of medical importance are known to occur. This study includes research pertaining to the development of methods for rearing large numbers of medically important arthropods. Two species in particular, *Xenopsylla cheopis*, the oriental rat flea, and *Triatoma barberi*, a reduviid bug, have been added to our present colonies of six mosquito species, a sand fly, two tick and one other reduviid bug. The first species is the main vector of bubonic plague worldwide while *T. barberi* is a vector of chagas disease in Mexico. Data concerning repellent susceptibility or resistance with these species are unknown.

RESULTS AND DISCUSSION OF RESULTS

The oriental rat flea colony was started on 9 February 1979, from stock sent by Dr. R. H. Roberts of the Insects Affecting Man Research Laboratory in Gainesville, Florida. Blood feeding is accomplished with suckling mice and a thriving colony has been the end result. A repellent test system using white mice has been initiated to obtain repellent data on the fleas.

The *Triatoma barberi* colony was obtained from Lauren Zarate at the G. W. Hooper Foundation on 8 June 1979. This particular colony is presently being built up to higher levels in expectation of repellent testing.

CONCLUSIONS

A large and comprehensive selection of test species has been established and is being maintained at production levels for repellent research. Two new species will be tested as time permits to obtain information and new insights into the mechanisms of insect repellency.

RECOMMENDATIONS

Since different arthropod species will show wide variation in reactions

to present repellents, it is advisable to obtain and test as many insect species as possible. Data analysis and correlation of these varied results will better our understanding on the nature of repellency.

PUBLICATIONS

RUTLEDGE, L.C., M.A. LAWSON, L.L. YOUNG and M.A. MOUSSA. Rearing of *Diamanus montanus* (Siphonaptera: Ceratophyllidae) and *Hoplopsyllus Anomalus* (Siphonaptera: Pulicidae). J Med Entomol 15:304, 1979

STUDY NO. 2 Entomological evaluation of insect repellents

PROBLEM

Insect-borne diseases are among those of paramount importance to military forces in the field. Insect repellents are among the only practical and effective means available for preventing insect-borne diseases in field units operating in areas or situations where reduction of insect populations is impracticable. This report focused on three aspects of this problem. First, an insect repellent survey was conducted at 7 Army posts. Information concerning efficacy and troop acceptability of insect repellents currently issued in the field is practically nonexistent. While consumer feedback constitutes an important segment of repellent research in private industry, the military, up to this point, has neglected to collect data in this area. Second, diethyl toluamide is issued to Army units as a 75% concentration in both lotion and aerosol forms. This concentration has been criticized as being too high for optimal safety and consumer acceptability. Up to this time, it has not been known to what level the concentration of diethyl toluamide in the solution could be reduced without lowering the repellent activity on the skin to an unacceptable level.

The development of more effective mosquito repellents currently demands the screening of large numbers of chemicals. In addition, synthesizing new polymer formulations of standard repellents can greatly reduce loss of repellent efficacy overtime. The selection and testing of the most promising compounds and new formulations are reported here.

RESULTS AND DISCUSSION OF RESULTS

In our insect repellent survey a total of 7 installations were surveyed, 3 TRADOC and 4 FORSCOM, and 1542 questionnaires were tabulated. Results of the more pertinent questions indicated that a majority,

(42.3%) said the current Army repellent is effective for 1 hour or less, and 82% said it was effective for 3 hours or less. The formulation of preference was spray (52%), cream/lotion (16.6%), and liquid (13.3%).

Fifty-nine percent claimed they used a commercial repellent even when Army issue was available. When asked if the Army needs a better repellent, 65.1% answered affirmatively.

In determining the optimal field strength of deet, we estimated the following relationship to indicate the amount of repellent necessary to maintain a 95% or better level of protection over a given period of time against *Aedes aegypti*.

Aedes aegypti/diethyl toluamide

0.16 mg/cm^2 deet	provide	0.0 hours of 95%+ protection
0.20		2.2
0.40		2.8
0.60		3.2
0.80		3.4
1.0		3.6
1.2		3.8
1.4		3.9
1.6		4.0
1.8		4.1
2.0		4.2
2.2		4.3
2.4		4.4

It has been calculated that the amount of repellent applied by the average person is 0.74 mg/cm^2. At approximately 1.0 mg/cm^2 the skin becomes saturated and application of any more repellent becomes redundant as well as cosmetically unacceptable.

The screening of approximately 120 compounds supplied by the Stanford Research Institute and the Department of Agriculture has led to the selection of 10 compounds found to be the most promising. Two compounds SRI 835-23A (N-(n-octyl)-glutarimide) and SRI 165-67 (N-(n-hexyl) -2-oxazolidine) have been advanced to the acute-phase toxicology stage of development.

Twenty-eight polymer formulations of diethyl toluamide, ethyl hexanediol, and sulfonamide were tested for repellent efficacy with an in vitro test system. Approximately 22 of these compounds indicate equal

or greater repellent efficacy than the unformulated material. Further testing using animals is in progress.

CONCLUSIONS

The results of the insect repellent survey indicate that the majority of Army personnel find the current field issue repellent to be inadequate or cosmetically inferior to commercial preparations. Other data suggest that the concentration of diethyl toluamide currently issued may be lowered with no corresponding loss of protection. Ten compounds and 22 formulations have shown particular promise as improved repellents. Further testing is warranted to fully evaluate these chemicals.

RECOMMENDATIONS

Since the active ingredients in the Army issue repellent are the same, and in fact of greater strength than commercial varieties, the poor acceptability of the Army product is due in part to commercial "hype" rather than lack of effectiveness. Recommend increased education of military personnel regarding use of current military issue repellents.

We suggest also that the current concentration of diethyl toluamide be lowered to 50%. The advantage of this would be lower cost as well as greater consumer acceptability.

Finally, we recommend continued testing of the formulations and compounds selected to be most promising.

PUBLICATIONS

RUTLEDGE, L.C., R.K. SOFIELD and M.A. MOUSSA. A bibliography of diethyl toluamide. Entomological Society of America Bulletin, 24:431-439, 1978

SKINNER, W.A., H.T. CRAWFORD, L.C. RUTLEDGE and M.A. MOUSSA. Topical mosquito repellents. XI: Carbamates derived from N,N^1-disubstituted diamines. J Pharm Sci, 69:390-391, 1979

SKINNER, W.A., H.T. CRAWFORD, L.C. RUTLEDGE and M.A. MOUSSA. Topical mosquito repellents. XII: N-substituted ureas and cyclic ureas. J Pharm Sci, 68:391-392, 1979

SKINNER, W.A., F. FUHRMANN, L.C. RUTLEDGE, M.A. MOUSA and C.E. SCHRECK. 1980. Topical mosquito repellents. XIII: Cyclic analogs of lactic acid. J Pharm Sci (in press)

Development of Improved Insect Repellents for Military Use

STUDY NO. 3 Development of improved tick repellents for military use

PROBLEM

Military personnel are often required to operate in areas known to be endemic for tick-borne diseases. It is often impractical, if not impossible, to treat such areas with insecticides prior to use by combat troops. One method to counter this problem is the use of effective tick repellents. The current standard repellents are not totally effective against ticks.

Various species of *Ornithodoros* ticks are vectors of relapsing fever in North America, the Middle East, Central America and elsewhere in the world. Little is known of their sensitivity to insect repellents in common use. Repellent testing against the Argasid tick *Ornithodoros parkeri* was continued this year.

RESULTS AND DISCUSSION OF RESULTS

In FY 1979, ED_{50} of three standard repellents were determined for nymphs of the Argasid tick *Ornithodoros parkeri*. Results indicate dimethyl phthalate to be markedly better than diethyl toluamide in tests against this tick. Ethyl hexanediol was greatly inferior to both of these compounds.

CONCLUSIONS

Dimethyl phthalate was the best of 3 standard repellents tested against *Ornithodoros parkeri* nymphs. Nymphs, larvae and adults can exhibit different susceptibilities to a repellent.

RECOMMENDATION

Repellent testing against *Dermacenter* and *Ornithodoros* ticks should be to identify the most effective overall repellents in consideration of genus and stage of the life cycle.

PUBLICATIONS

None

STUDY NO. 4 Development of duration testing methods for mosquito repellents

PROBLEM

While many chemicals are known to repel mosquitoes effectively when

applied to the skin at practical dosages, relatively few persist on the skin for extended periods of time after application. Persistence is an important property since military operations in which continuous prolonged exposure of personnel to mosquitoes may preclude frequent application. Conventional methods are inadequate for unequivocal determination of the persistence properties of new mosquito repellents. Quantitative statistical methods based on modern bioassay techniques are critically needed in the repellent development program.

RESULTS AND DISCUSSION OF RESULTS

In accordance with current efforts to minimize the use of human subjects in scientific research, test systems utilizing white mice and dogs were developed and refined in addition to developing safe and standardized test methods for humans.

The white mouse test system is used to determine the ED_{50} and the 4-hour ED_{50} of a particular test repellent. The 4-hour ED_{50} provides a measure of the combined effects of the intrinsic repellency and the persistence properties of the material under test on its efficacy as a repellent.

In the 4-hour ED_{50} test procedure, suckling mice are treated by immersion in ethanol (control) and 4 serial dilutions of the test repellent in ethanol. The treated mice are held separately in an incubator at 37 C for 4 hours and then are transferred to individual compartments in a cage containing test mosquitoes. The numbers of mosquitoes feeding on each mouse are recorded at 2-minute intervals for 20 minutes, and the totals of the 10 counts made on each mouse are recorded as the test results. In subsequent replicates, the range of dosages used is adjusted as necessary to bracket the 50% end point. The 4-hour ED_{50} of the test repellent is calculated by probit analysis of the results obtained in tests that bracket the 50% end point.

The dog repellent test system is similar. A template is used to mark off 5 equal size circles on the side of the animal, 1 control (ethanol) and 4 serial dilutions. A plastic cage with 5 holes to match the template and filled with the test insects is strapped to the side of the animal. Zero-hour or 4-hour tests can be conducted by putting the cage on the animal at the appropriate time and then counting the insects feeding after 90 seconds. Data analysis is carried out in the same manner as the white mouse system.

The human test system is organized as follows. Five circular test areas are outlined with a template on the flexor region of the forearm and treated with ethanol (control) and four serial dilutions of the test repellent. A cage having matching cutouts on the floor and

containing 15 mosquitoes is placed on the forearm, and the numbers of mosquitoes feeding on the control and the repellent treatments are recorded. In subsequent trials, the range of dosages applied to the forearm is adjusted to bracket the median effective dosage (ED_{50}) of the test repellent. The test is replicated at that range of dosages until a valid estimate of the ED_{50} can be obtained. The data are analyzed similarly to the white mouse and dog test system. This method for evaluating mosquito repellents on human volunteers has been submitted to the American Society for Testing and Materials (ASTM).

CONCLUSIONS

New repellents should be screened and evaluated on experimental animals prior to testing on humans. The established principles of biological assay are applicable in testing repellents in animal and human test systems.

RECOMMENDATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6300	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A802	00	121
b. [redacted]	62772A	3M162772A810	00	016 AFC 504A
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Diagnosis and Prevention of Leishmaniasis in Military Personnel

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

0010100 Microbiology; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	79 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: Not Applicable d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 79	2.6	92
CURRENT FISCAL YEAR 80	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. academic institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Antigen; (U) Diagnosis; (U) Immunity; (U) Disease Vector; (U) Leishmania; (U) Serology; (U) Epidemiology

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop improved methods for diagnosis and prevention of cutaneous leishmanial infections encountered by service personnel deployed or stationed in endemic areas overseas. The absence of protective vaccines and lack of satisfactory treatment renders these studies essential to protection of the health of field soldiers.

24. (U) Mass culture of *Leishmania* will be instituted to provide sufficient cells for biochemical isolation of cellular components. Immunochemical analysis will be conducted to define components which offer greater sensitivity and specificity for use in serodiagnosis and skin tests. In vivo immune responses to purified components and in vitro immunochemical reactions will be conducted to characterize humoral and cell mediated mechanisms and to evaluate antigens for potential protective value against infection.

25. (U) 78 10 - 79 09. *L. braziliensis* promastigote homogenate extract was used as antigen in the enzyme-linked immunosorbent assay (ELISA) to measure antibody in sera from human with American cutaneous leishmaniasis. Antibody was detected in 61% of the cases. However, reactions were also positive with sera from 50% of TB patients, 70% of malaria patients and 100% of trypanosomiasis, shistosomiasis, echinococcosis and filiarisis patients. ELISA determined significant increases of antibodies to human blood group antigens and BSA in rabbits immunized with *L. braziliensis* antigen. The data suggest that cross reactions between *Leishmania* and other parasites partly result from shared host and/or culture medium antigens. Therefore, application of ELISA to serodiagnosis of leishmaniasis is dependent on production and use of highly specific antigens. This work unit is terminated because the mission has been transferred.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3M162772A810 Military Skin Disease

WORK UNIT NO. 016 Diagnosis and Prevention of Leishmaniasis in Military Personnel

The following investigation has been conducted under this work unit:

STUDY NO. 1 Serodiagnosis of American leishmaniasis

The response to immunization against *L. braziliensis* was followed in rabbits by measuring antibody by enzyme-immunoassay (ELISA), passive hemagglutination (PHA), complement fixation (CF), and counter-current immunoelectrophoresis (CCIE). PHA and CF titers were interpreted according to standard methods, whereas ELISA exact titers were derived by the equation Y = a-b logX which describes the straight line that results when absorbances are plotted against test serum dilutions. Peak titer was measured at 32±1 days after initial injection, irrespective of the assay method. Seven days post-infection antibody titer was 1130 by ELISA, 0 by PHA and 8 by CF. Precipitin bands were shown by CCIE only at peak titer. With sera from hamsters experimentally infected with *Leishmania*, 94% showed leishmanial antibody by ELISA, compared to 92% by CF and 65% by PHA. Serum samples from 31 human patients with cutaneous leishmaniasis were tested only by ELISA. Of these, 23 were positive for leishmanial antibodies.

BODY OF REPORT

WORK UNIT NO.	016	Diagnosis and prevention of Leishmaniasis in military personnel
STUDY NO.	1	Serodiagnosis of American leishmaniasis

PROBLEM

Standard serological methods are not sensitive and/or specific enough to detect low-level circulating antibodies in sera of patients during or after infection with *Leishmania*. Thus, treatment is delayed until the appearance of a distinct lesion from which a positive diagnosis can be made. The development of a simple and reliable method for the serodiagnosis of leishmaniasis will permit early treatment, thereby decreasing complications which may arise from mucocutaneous or visceral involvement. The introduction of enzyme-linked-immunosorbent assay (ELISA) provided a method which was investigated for its potential usefulness in the serodiagnosis of American cutaneous leishmaniasis. Quantitative antigen-antibody relationships in ELISA were established and defined to arrive at a method for the assessment of leishmanial antibody in the sera of military personnel serving in, or returning from endemic areas. However, non-specific reactions need to be defined in order to arrive at an accurate and reproducible assessment of leishmanial antibody in the sera of immunized and/or infected subjects. This study was designed to modify established serodiagnostic methods and/or develop new methods for the early detection of leishmanial infections.

RESULTS AND DISCUSSION OF RESULTS

Patient's antibody titers were quantified by incubating serial two-fold serum dilutions in microtiter plates coated with leishmanial antigen. The antigen consisted of clear supernatant from a homogenate prepared by freeze-thawing and sonicating promastigotes of *L. braziliensis*. Conjugate was rabbit anti-human immunoglobulin labeled with alkaline phosphatase. One hour after the addition of substrate, the reaction was stopped and absorbances were read at 405 μm with a Coleman Jr. spectrophotometer. Previous studies from this laboratory established that increased absorbances in ELISA represent conjugated antiglobulin-leishmanial antibody ratios. Thus, when absorbances are plotted against test serum dilutions, a straight line results which follows the equation $y=a-b \log X$, where a is the intercept of the y (absorbance axis, b is the slope of the line, and x is the serum dilution). The data were applied to this equation by deriving a and b for three or more absorbances obtained in each serum titration and then adjusting all end point titers to 0.01 absorbance. ELISA baseline values

for serial two-fold dilutions of sera from normal humans were subtracted from corresponding dilutions of test sera before final calculations were made.

Serum from 8 of 13 cases of cutaneous leishmaniasis reacted with *L. braziliensis* antigen in micro-ELISA with titers ranging from 1:80 to 1:2572. Five of 7 trypanosomiasis cases were positive titers of 1:80 to 1:7831, seven out of 10 cases of malaria were positive with titers of 1:10 to 1:10240, 12 of 23 cases of tuberculosis were positive with titers of 1:10 to 1:10240. Two cases of syphilis and *T. saginata* - were negative, while one case of echinococciasis and one case of schistosomiasis reacted with titers of 1:2560.

CONCLUSIONS

The results show the extreme sensitivity - and usefulness of ELISA for the sera diagnosis of American cutaneous leishmaniasis.

RECOMMENDATIONS

Further study is required to associate false negative reactions with such factors as time course of disease. False positives and cross reactions will be greatly minimized with identification and purification of specific antigen.

PUBLICATIONS

LUZZIO, A.J., M.J. McROBERTS, and N.H. EULISS. Quantitative estimation of leishmanial antibody titers by ELISA. J Infect Dis 140:370-377, 1979

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OE 6313	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A810	00	017 APC 504A
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

11. TITLE (Procede with Security Classification Code)

(U) Area Repellents for Collective Protection of Troops from Vector-borne Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

005900 Environmental Biology; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 10	79 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: Not Applicable d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	3.1	45
FISCAL YEAR CURRENT 80	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Rutledge, L.C., M.S., DAC

NAME: Semey, Howard G., SP6, AMEDD POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Repellent (U) Mosquito (U) Disease (U) Prevention (U) Formulations (U) Dispersal Equipment (U) Vector (U) Protection of Troops

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The protection of troops from vector-borne diseases is essential to the maintenance of an effective combat force. Skin repellents currently available for use by military personnel are not totally effective nor readily acceptable by the users. The proposed study is designed to test and evaluate repellents for field application in providing collective protection of troops in areas of intense activity.

24. (U) Candidate chemical compounds suitable for area repellency yet compatible with the environment will be identified; formulations appropriate for field dissemination will be made; dispersal equipment will be selected and evaluated for field use; repellent formulations will be field tested and their rates of application, biological effectiveness, duration of protection as well as economy of operation determined.

25. (U) 7810-7909. Laboratory area repellent testing procedures were developed and testing cages designed and constructed. Twenty-one selected materials were tested for area repellency in the laboratory against the yellow fever mosquito, *Aedes aegypti*. Citronellal, geraniol, allethrin, D-trans-allethrin, N,N-diethyl-m-toluamide, a petroleum oil fraction, and Mosquito Beater^{R}, a commercial area repellent, exhibited the best area repellency and warrant continued laboratory testing. The petroleum oil fraction was distilled and distillate cuts exhibiting the greatest repellency were pooled and fractionated by liquid chromatography. The most active fractions were subjected to gas chromatographic-mass spectrophic analysis, and 1-methyl-naphthalene (1-MN) and 2-methyl-naphthalene (2-MN) were identified as the major components. Commercial samples of 1-MN and 2-MN exhibited excellent area repellency in the laboratory. Field tests using these materials have been initiated. Terminated and consolidated with similar research in a new work unit.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3M162772A810 Military Skin Disease

WORK UNIT NO. 017 Area Repellents for Collective Protection of Troops from Vector-borne Diseases

The following investigation has been conducted under this work unit:

STUDY NO. 1 Testing and evaluation of area repellents for field application in providing collective protection of troops in areas of intense activity

Laboratory area repellent testing procedures were developed and testing cages were designed and constructed. Twenty-one selected materials were tested in the laboratory for area repellency against the yellow fever mosquito, *Aedes aegypti*. Citronellal, geraniol, allethrin, D-*trans*-allethrin, N,N-diethyl-m-toluamide, a petroleum oil-fraction, and Mosquito Beater^{R}, a commercial area repellent, exhibited the best area repellency and warrant continued laboratory testing. The petroleum oil fraction was distilled and distillate cuts exhibiting the greatest repellency were pooled and fractionated by liquid chromatography. The most active fractions were subjected to gas chromatographic-mass spectroscopic analysis, and 1-methyl-naphthalene (1-MN) and 2-methyl naphthalene (2-MN) were identified as the major components. Commercial samples of 1-MN and 2-MN exhibited excellent area repellency in the laboratory. Field tests using these materials have been initiated.

AD-A116 951 LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANC--ETC F/G 6/5
ANNUAL RESEARCH PROGRESS REPORT.(U)
SEP 79

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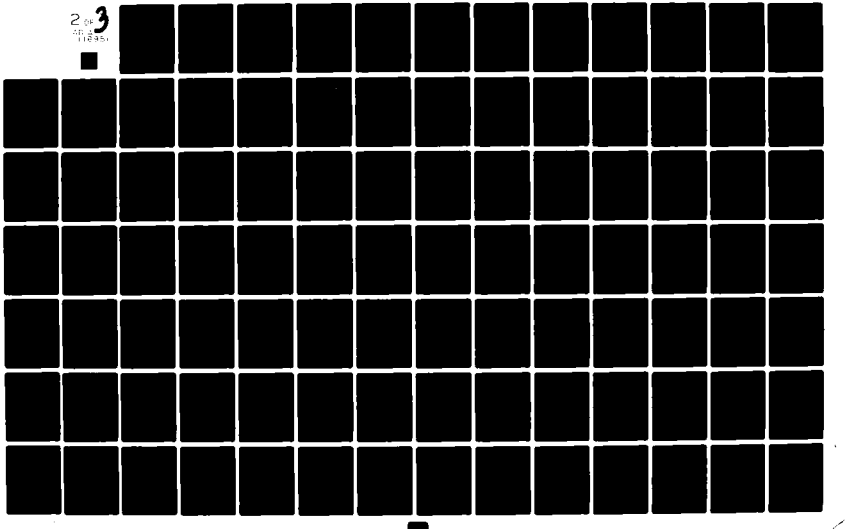

BODY OF REPORT

WORK UNIT NO.	017	Area Repellents for Collective Protection of Troops from Vector-borne Diseases
STUDY NO.	1	Testing and evaluation of area repellents for field application in providing collective protection of troops in areas of intense activity

PROBLEM

The need to protect the military population from vector-borne diseases is essential for the maintenance of an effective combat force. In overseas areas where troops are, or may be deployed and where endemic foci of disease exist, arthropod-borne diseases continue to be a major health problem to military forces. The use of repellents appears to be the most practical means of interrupting arthropod-borne diseases, especially those for which vaccines or chemoprophylactic drugs are not available. The development of an effective area repellent could protect troops in areas of increased personnel activity without the disadvantages and shortcomings of skin repellents currently available in the military supply system..

RESULTS AND DISCUSSION OF RESULTS

Laboratory area repellent testing indicates that the following compounds warrant further laboratory and/or field testing. Mosquito Beater^{R}, 1-methyl-naphthalene, 2-methyl-napthalene, citronellal, geraniol, allethrin, esbiol, D-*trans*-allethrin and deet. Area repellent field testing methodology was developed and the required equipment procured. A testing site was selected and testing coordinated with the Colusa county mosquito abatement district manager. Three successful field trials were completed during which Mosquito Beater^{R}, 1-methyl-napthalene, and 2-methyl-naphthalene were tested for efficacy against populations of *Culex tarsalis* and *Aedes melanimon* mosquitoes. The 96 light trap samples have been counted and the results are being compiled for statistical analysis.

CONCLUSIONS

Area repellent field test data for Mosquito Beater^{R}, 1-methyl-napthalene and 2-methyl-napthalene have not been analyzed statistically. However, examination of the raw data indicates that none of the three compounds tested is highly effective as an area repellent when using CO_2/light traps as an indicator of efficacy.

Area Repellents for Protection of Troops from Vector-borne Diseases

RECOMMENDATIONS

Current plans include the area repellent field testing of citronellal, geraniol, allethrin, esbiol, D-*trans*-allethrin, and deet. The testing schedule will depend on the availability of personnel and funding. Materials exhibiting effective area repellency will be subjected to duration testing in the laboratory and field. If none of the nine compounds tested prove effective, the area repellent program will be terminated.

PUBLICATIONS

WIRTZ, R.A., J.D. TURRENTINE and L.C. RUTLEDGE (1980) Mosquito area repellents: Laboratory testing of candidate materials against *Aedes aegypti* (L.). (Submitted for publication)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6116	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 07 02	TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A812	00	001 APC 506C
b. CONTRIBUTING				
c. [redacted]	CARDS 1141			

11. TITLE (Precede with Security Classification Code)

(U) Studies to Assure a Supply of Non-human Promates for Research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

001700 Animal Husbandry; 012900 Physiology; 002600 Biology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	79 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING	79	1.0	80
FISCAL YEAR — CURRENT	80	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

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20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
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PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. academic institution)

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ASSOCIATE INVESTIGATORS

NAME: Jones, A. SP5

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Primate; (U) Reproduction; (U) Animal Colony

(U) Physiology; (U) Behavior; (U) Husbandry

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) An assured supply of primates is essential for continued research to solve Army medical problems in infections disease, new drug development, and a variety of other areas. Curtailment of export of the Colombian source has limited the overseas supply of owl monkeys (Aotus) required for ongoing malaria research. Owl monkeys are nocturnal primates and there are few data on the requirements in captivity or on the behavior and needs of their offspring. This work unit will develop information on reproductive biology, nutrition, disease control and low-cost husbandry methods for domestic production of required owl monkeys.

24. (U) Owl monkey pairs will be matched by karyo-type and housed in a variety of cage/group arrangements. Reproductive potential will be evaluated by observation and physiologic measurements.

25. (U) 7809-7910. There are currently 113 animals in the owl monkey colony, 60 adult males and 53 adult females. During FY 79 there were 21 live births, 5 abortions, and 1 stillborn. Since the colony was established in May 1975, 5 progeny have been born. Basic husbandry conditions and practices have been established and hematological, biochemical, developmental, and behavioral data have been accumulated and analyzed. All animals have been karyo-typed and fecundity evaluated in terms of karyo-type matching and adaptation to laboratory conditions. Minimum conditions of operation of a laboratory breeding colony have been defined. Projects completed during FY 79 are as follows: 1) Behavior study on differences between 2 subspecies of Owl Monkeys, 2) report on production record of the LAIR colony, and 3) report on hybrid births of Owl Monkeys. The research mission of the Animal Resources Group, Division of Research Support, LAIR, was officially terminated 1 Oct 79. The colony of owl monkeys being utilized under this work unit were released to WRAIR and the work unit terminated 1 Oct 79.

*Available to contractors upon affiliation's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M162772A812 Military Research Animal Resources

WORK UNIT NO. 001 Studies to Assure a Supply of Non-Human Primates for Research

The following investigation has been conducted under this work unit:

STUDY NO. 1 Basic studies in reproduction of Owl monkeys

Non-human primates are essential for a number of areas of military biomedical research. Most notably they are used in infectious disease to establish pathogenesis of infections and tests of chemotherapeutic agents and vaccines. They are also needed for research in trauma, resuscitation, performance, and toxicology. All of these studies are designed to benefit military personnel who are exposed to disease or toxic substances, or who suffer injury in the performance of duty.

Ability to obtain non-human primates from foreign sources is decreasing; future supplies depend upon development of domestic breeding colonies. Much needs to be learned so non-human primates can be produced in large numbers as economically as possible.

This study has made substantial contributions to the limited amount of data available on the husbandry and reproductive physiology of this nocturnal primate.

STUDY NO. 1. Currently there are 122 monkeys in the colony, exclusive of unweaned offspring. There are forty-two breeding pairs which produced twenty-four viable offspring during FY 79. All animals have been karyotyped and fecundity evaluated in terms of karyotype matching and adaptation to laboratory conditions. Basic husbandry conditions and practices have been established and hematological, biochemical, developmental, and behavioral data have been accumulated. The research mission of the Animal Resources Group was officially terminated 1 October 1979. The colony of owl monkeys being utilized under this work unit was released to WRAIR on that date.

BODY OF REPORT

WORK UNIT NO. 001 — Studies to Assure a Supply of Non-Human Primates for Research

STUDY NO. 1 — Basic studies in reproduction of Owl monkeys

PROBLEM

There is a critical shortage of Owl monkeys (Aotus Sp.) for biomedical research because the countries of origin have severely limited exportation. In the near future, Owl monkeys will be placed on the endangered species list. This will make importation of these animals impossible at any cost.

Owl monkeys are the only non-human primate in which falciparum malaria can be maintained. Consequently, the Army is dependent upon these animals for research in prevention and treatment of malaria.

The long-term solution to provide a source of these animals is a domestic breeding program. Little is known about the reproductive biology or behavior of Owl monkeys. Until recently, consistent breeding and rearing of these animals in captivity have not been accomplished.

The objectives of this study are to determine the optimal environmental conditions for domestic in-house breeding, husbandry procedures, and nutritional requirements of the Owl monkeys. The animals will be studied extensively through the life cycle. Data will be accumulated which will reflect karyotyping, pregnancy diagnosis, growth and behavioral patterns, the blood chemistries and urine analyses. All animals will be seen at necropsy after they have expired and the tissues will be examine- histopathologically.

RESULTS AND DISCUSSION OF RESULTS

At the termination of this work unit there were 122 monkeys in the colony. This is exclusive of ten unweaned offspring. Since the arrival of the first group of Owl monkeys in May 1975, the breeding colony has produced 75 live progeny. Twenty-four offspring were born during FY 79. The colony contains 44 wild-caught males, 35 wild-caught females, 27 LAIR-born males, and 16 LAIR-born females.

The first animal produced by LAIR-born parents was born 31 July 1979. The age of the father was 3 years, 7 months, and the mother was 2 years, 10 months. Three additional LAIR-born parents have produced viable offspring subsequently. The first offspring of Bolivian-origin parents,

karyotype VI, was born on 9 January 1979. Two additional offspring were produced, one to the parents which produced the first Bolivian-origin offspring and one to another set of Bolivian-origin parents. A total of four hybrid offspring were produced. One pair, karyotype III x karyotype IX, produced three viable offspring; and another pair, karyotype II x karyotype VIII, produced one offspring.

A total of 16 juvenile animals were kayotyped during FY 79. The cytogenetic analysis was performed by Dr. T.C. Jones, Pathobiology, Inc., Marlboro, MA. Nine males and seven females were typed and four karyotypes and one hybrid cross were identified. Four animals were shown to be karyotype II, three karyotype IV, seven karyotype II, one karyotype IX, and one hybrid cross-karyotype III and IX.

Weaning of animals has been the area of concern in terms of colony management during FY 79. Previously, young animals were totally separated from their parents at four months of age and placed with one or more peers. This proved to be extremely stressful to several of the young whose deaths were associated with trauma. During FY 79, animals were weaned at five months of age and placed alone in a small cage adjacent to the parents' cage for a minimum of one week. They were then placed in larger cages with peers. These animals adjusted to parent separation rapidly and completely.

There were 17 deaths during FY 79, including 7 adults and 10 LAIR-born young. All animals were necropsied and tissues collected for histopathological examination. Causes of deaths in young animals have been associated with septicemia at or near parturition, stress of weaning, and parental rejection or abuse. In adult animals, cause of death has been most often associated with bacterial infections; the other deaths have been attributed to a variety of causes. For example, one adult died as a result of severe hemorrhage after amputation of a toe. This animal exhibited signs of a defective clotting mechanism.

Of several proposed projects, only behavioral studies have been feasible, due to the loss of funding for the colony. A study comparing the behavioral differences between monkeys of Colombian and Bolivian origin was completed, and a paper outlining the production and progress of the colony from May 1975 to March 1979 was written. A report on the birth of hybrid offspring is in preparation.

CONCLUSIONS

Wild-caught Owl monkeys of Colombian and Bolivian origin, as well as the laboratory-born animals can be maintained and bred effectively in a controlled environment.

Basic studies in reproduction of Owl monkeys (cont'd)

Husbandry conditions in the LAIR colony are appropriate and efficient in terms of fecundity.

Potential mates must be matched by karyotype for maximum production.

RECOMMENDATIONS

The breeding and maintenance of the Owl monkey colony should be continued and the physiologic requirements for reproduction of laboratory-born animals should be delineated.

PUBLICATIONS

Publications reflecting the research under this work unit were listed in the LAIR Annual Research Progress Report, 1978, p 268.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OE 6114	2. DATE OF SUMMARY* 79 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 79 08 15	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DoD'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	061 APC 506E
b. CONTRIBUTING	62772A	3M162772A812	00	005
c. CONTRIBUTING	CARDS 114f			

11. TITLE (Precede with Security Classification Code)*

(U) Disease Mechanisms at the Cellular Level

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 010100 Microbiology; 002300 Biochemistry; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
76 10	Cont	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	0.8	50
	CURRENT 80	3.0	59

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Histochemistry; (U) Electron Microscopy; (U) Diagnosis; (U) Infection; (U) Laboratory Animal; (U) Metabolic Disease

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Prevention and control of disease depends upon complete understanding of abnormal processes involved, from initial cellular injury to repair. Providing pathology support to LAIR's varied research program requires continued development of highly specialized techniques and accurate diagnosis of spontaneous disease in experimental animals. This project will develop improved methods to support LAIR investigators. It will provide information on cellular response to injury and differentiate naturally-occurring disease from experimentally-induced lesion.

24. (U) Improved techniques for pathology support will be developed and tested. Histopathology, histochemistry, electron microscopy and quantitative analytical methods will be used. Accurate diagnoses of diseases in LAIR's laboratory animals will be sought in order to provide quality control, efficient management, and accurate interpretation of experimental results.

25. (U) 7908-7910. Due to realignment of mission and organization, the direction of this work unit changed in FY 79. Buffer systems with electrolyte composition, pH and osmolarity matching that of liver, kidney, and retina were made and tested as reagents in preparation of these tissues for electron microscopy. Histochemical stains to demonstrate iron and fungi in plastic embedded tissues were developed. Evaluation of a method using thiocarbohydrazide and osmium tetroxide for preparation of tissues for scanning electron microscopy was begun. Reports and interpretation of complete necropsy and histological examination of colony animals dying of naturally-occurring disease were provided to investigators concerned.

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M162772A812 Military Research Animal Resources

WORK UNIT NO. 005 Disease Mechanisms at the Cellular Level

The following investigations have been conducted under this work unit:

STUDY NO. 1 Development of improved histological, histochemical and ultrastructural techniques for study of experimentally induced and naturally occurring diseases of laboratory animals

UNNUMBERED STUDIES Reported under work units of other LAIR investigators

Prevention and control of disease, documentation, and accurate interpretation of experimental results in laboratory animals depend upon a complete understanding of abnormal processes involved from initial cell injury to repair. Providing pathology support to LAIR's varied research program requires continued development and modification of highly specialized techniques and accurate diagnosis of spontaneous disease in experimental animals. Three special techniques were studied, modified, and evaluated: (1) an improved buffer system for preparation of tissues for electron microscopy, (2) a special staining procedure for plastic embedded tissues used for autoradiography of lipid soluble components, (3) a special staining procedure for demonstration of iron in tissues embedded in glycol methacrylate.

The buffer system was designed to match physiological concentrations of electrolytes, osmolarity, and pH in heart muscle, skeletal muscle, spleen, liver, kidney, and retina. Preliminary evaluation indicates that this buffer system, when combined with glutaraldehyde and paraformaldehyde, gives excellent results in all tissues tested except retina.

Adaptation of the Perl's reaction to methacrylate embedded spleen and bone marrow sections sectioned at a thickness of 1 μm provided greatly improved resolution compared to results in paraffin embedded tissue. This technique improves the ability to localize and estimate relative amounts of iron in tissue sections.

BODY OF REPORT

WORK UNIT NO. 005 — Disease Mechanisms at the Cellular Level

STUDY NO. 1 — Improved histological, histochemical, and ultrastructural techniques for study of experimentally induced and naturally occurring diseases of laboratory animals

PROBLEM

Prevention and control of disease, documentation, and accurate interpretation of experimental results in laboratory animals depend upon complete understanding of abnormal processes involved from initial cell injury to repair. Providing pathology support to LAIR's varied research program requires ongoing development and adaptation of highly specialized techniques designed specifically to support Institute projects. Many projects provide unique challenges and specific problems in demonstrating and interpreting pathologic changes in tissue. Three special techniques were developed during FY 79 which promise to improve pathology support capabilities for current and future Institute projects.

Fixation and initial processing of tissues are among the most important procedures in electron microscopy. Failure of these procedures renders subsequent results useless or perhaps misleading. A perfect buffering system and fixation technique that is universally applicable to all tissues does not exist and can never be developed because of the differences in tissue composition. To overcome some of these difficulties a buffer system was developed in which the electrolyte content, pH, and osmolarity were matched with those of rat liver, kidney, and retina. These buffers were used as reagents and washes in the tissue harvesting process and later were combined with 3.0% glutaraldehyde and 2% paraformaldehyde as a primary fixative for these tissues. Blocks of fresh tissue measuring 1 cu mm were immersed in the fixative-buffer solution. Oxygen (95%) was bubbled through the solution for the first 5 min. Tissues were allowed to remain in the primary fixative for 2 h and were dehydrated and embedded in Epon/Araldite by routine procedures. Sections 1 µm thick were cut, stained, examined, and evaluated by light microscopy.

Another technique developed, tested, and put into routine use was a staining method for tissue sections embedded in plastic and coated with photographic emulsion for light microscopic autoradiography. Previously used staining procedures resulted in one or more of the following problems: fading of the stain, leaching of lipid soluble tissue components, chemical activation of the light emulsion, and removal of the silver

halide granules by oxidation. A staining procedure, in which methylene blue, azure II, and basic fuschin are used as the primary ingredients, was applied to developed autoradiographic sections. In these sections, ^{3}H-labelled, 11,12 retinyl acetate (a lipid soluble vitamin) was demonstrated.

Embedding tissues in water soluble plastic permits much greater resolution and fewer artifacts than conventional paraffin embedding. Many of the routine staining procedures can be adpated to tissue embedded in glycol methacrylate and sectioned at a thickness of 1 μm. Methods for demonstration of iron are particularly valuable in experiments dealing with iron metabolism in which accurate localization and estimation of relative quantities of this element are necessary.

RESULTS AND DISCUSSION OF RESULTS

The buffer system based on physiological concentrations of electrolytes appears to be promising. Results in kidney, liver, spleen, and myocardium fixed with this buffer were excellent. Sections of retina contained minute vacuoles which are probably artifact. Additional work is necessary to develop optimal buffers for retina and to evaluate results in other tissues.

The polychrome staining technique for epon-embedded tissues was sucessful, both as a stain for routine examination of 1 μm-thick sections and for autoradiography. No fading of the stain or chemography occurred. Lipids and lipid soluble substances were not removed by this technique. Autoradiographic demonstration of the lipid soluble vitamin had excellent quality.

Adaptation of the Perl's stain for iron to glycol methacrylate-embedded sections of spleen yielded excellent results. With the use of this technqique, it was possible to compare the stainable iron content in groups of animals fed different amounts of dietary iron. Preliminary results of this experiment are being evaluated and a larger experiment is currently being conducted.

CONCLUSIONS

Buffering systems based on physiological concentrations of electrolytes is a promising technique which may greatly improve fixation of tissues for electron microscopy. A polychrome stain (methylene blue, azure II, and basic fuchsin in epoxy-embedded tissue) is an ideal technique for use with light microscopic autoradiography. Those stains adapted to methacrylate-embedded tissue sections improve localization of iron and permit semi-quantitative comparisons.

Disease mechanisms at the cellular level (cont'd)

RECOMMENDATIONS

These techniques should be refined and improved and made part of routine procedures available for pathology support to Institute research projects.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6110	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. SMI'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM / A. WORK UNIT
79 08 15	D. Change	U	U	NA	NL	☒ YES ☐ NO	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	058 APC 504J
b. CONTRIBUTING	62772A	3M162772A812	00	024 APC 504J
c. CONTRIBUTING	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Neurobehavioral Investigations of Military Trauma

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support; 012900 Physiology; 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10 01	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR — PRECEDING: 79	1.1	31
FISCAL YEAR — CURRENT: 80	1.1	36

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Pribyl, V.J. DAC

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Neurophysiology; (U) Resuscitation; (U) Psychopharmacology; (U) Psychophysiology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code.)

23. (U) Resuscitation from trauma in combat operations imposes problems not typically encountered in civilian medical operations. Among these are the occurrences of mass casualties, and physical limitations of field medical facilities. Short, violent conflicts will further limit field medical support of combat operations and medical casualties may be required to continue limited support of combat operations. There will be greater emphasis on methods of treating combat injuries which permit rapid return to duty. The objectives of this work unit are to assess the effects of military trauma on combat effectiveness and to evaluate functional recovery following the use of different resuscitating procedures.

24. (U) The methods used emphasize the detection and quantification of those functional changes which could limit adaptation to the environment following trauma. Both behavioral and neurophysiological techniques are employed in order to increase the chances of detecting effects which are of functional significance to the organism. Behavioral testing is used to evaluate basic sensory and motor processes as well as more complex cognitive processes. The additional data obtained through appropriate analysis of spontaneous and evoked electroencephalographic activity provides furhter evidence of possible changes in central nervous system functioning.

25. (U) (7810-7910). Rats were transfused with Plasmate (5 percent solution of human plasma protein fraction). Hematocrit levels indicated replacement of 50 percent and 70 percent in two groups. Psychometric performance testing indicated low scores in the groups immediately following transfusion with gradual return to baseline levels by the tenth post-treatment day.

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M162772A812 Military Research Animal Resources

WORK UNIT NO. 024 Neurobehavioral Investigations of Military Trauma

The following investigations have been conducted under this work unit:

STUDY NO. 1 Dark adaptometer prototype

STUDY NO. 2 Field portable dark adaptometer

STUDY NO. 1. The early dark adaptometer prototype continues to be evaluated in terms of dark adaptation testing done under a previously approved human use protocol. In this initial protocol, 50 volunteers were tested. The results of these tests were statistically analyzed point-by-point to yield a mean curve and two standard deviation envelope functions.

STUDY NO. 2. A 36-inch Gonzfeld hemisphere with adaptation light and some connecting hardware was adapted to a Heath H-8 microcomputer. Hardware and software modifications were initiated to provide a real time chart (plot) of dark adaptation for ready evaluation of visual function. A human use protocol for testing of this new version was initiated.

BODY OF REPORT

WORK UNIT NO.	024	Neurobehavioral Investigations of Military Trauma
STUDY NO.	1	Dark adaptometer prototype

PROBLEM

The eye's ability to adjust from a very bright light to a very dim light environment is known as dark adaptation. The lack of this ability may be a congenital deficit or the ability may be severely altered by a disease (e.g., retinitis pigmentosa). Active duty soldiers may be not aware of their inability to "see" at night. These soldiers may be in command of companies or platoons in night maneuvers. Unintentionally, these persons may jeopardize the lives of other military personnel and allies, and destroy equipment. The magnitude of this dark adaptation problem in the military, specifically with the active duty fighting soldier, has not been fully documented. Presently, dark adaptometers are complex optical devices.

RESULTS AND DISCUSSION OF RESULTS

The early form of dark adaptometer, used to test 50 volunteers, consisted of a Gonzfeld hemisphere, adapting light, and a matrix of red and green light emitting diodes (LED). The output and control were derived from a Data General Nova 312 microcomputer. Permanent records were made by photographing the face of the CRT on the computer. The original device exemplified some major problems: the device was cumbersome to relocate; the software control was prone to frequent failure; and the minicomputer was subject to frequent failure. Reports on the original data illustrated a trend for "normal" eyes for both red and green test LEDs. To date, the current investigators have not been able to locate in the computer the original data from which early statistics were derived; therefore, precise statistical comparisons have not been made. A set of negatives indicating a mean and standard deviation for the red and green test targets was used for comparison purposes only.

CONCLUSIONS

While the computer-operated dark adaptometer test is important to the military, the original device requires considerable modifications for military use in the field.

RECOMMENDATIONS

A new, simpler device, microcomputer controlled, with a direct hard copy output, should be constructed.

PUBLICATIONS

None

STUDY NO. 2 Field portable dark adaptometer

PROBLEM

A new type of dark adaptometer has been developed at Letterman Army Institute of Research (LAIR) which is a piece of apparatus considerably less complicated than other dark adaptometers. Interface with a low-cost microcomputer system allows clinical flexibility for routine military screening and research flexibility for investigators studying the role of dark adaptation in military tasks.

RESULTS AND DISCUSSION OF RESULTS

The LAIR adaptometer is controlled by a low-cost microcomputer system. The computer controls all phases of test administration and data reduction. Several different test procedures are available to the operator through selection of program options. These include control over the duration of the light and dark adaptation periods, selection of the psychophysical test method (ascending limits or tracking), optional use of an automatically adjusted fixation point, and optional pre-filtering of the time series of threshold estimates before plotting. Provisions have also been made for selectively activating portions of the stimulus display and for varying the flash rate of the LED elements above and below the critical flicker fusion frequency. The microcomputer also organizes the subject's response data, calculates threshold estimates, and generates separate plots of the red and green dark adaptation functions. The digital records of the threshold estimates of each subject are also labeled and stored on magnetic disks for later statistical analyses.

CONCLUSIONS

The dark adaptometer, developed at LAIR, appears feasible for field use. We have now initiated a human use protocol for further testing.

RECOMMENDATIONS

Following data collection and analysis from volunteers at LAIR, four similar devices should be fabricated by USAMRDC for loan to MRDC and HSC laboratories to validate the utility of the device.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6087	79 10 01	DD-DRAE(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GROUP	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	004 APC 505N
b. CONTRIBUTING				
c. XXXXXXXXXXXX	CARDS 114f			

11. TITLE (Provide with security Classification Code)

(U) CPD-Adenine Clinical Trials

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 008800 Life Support

13a. START DATE	13b. ESTIMATED COMPLETION DATE	14. FUNDING AGENCY			15. PERFORMANCE METHOD
75 01	cont.	DA			C. In-House

16. RESOURCES ESTIMATE

		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 79	0.4	09
	CURRENT 80	1.0	24

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

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RESPONSIBLE INDIVIDUAL

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19a. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Moore, Gerald L., Ph.D., DAC

NAME: Sohmer, Paul R., CPT, MC POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Blood Storage; (U) Military Blood Banking; (U) red cell survival; (U) adenine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The final objective of this study, clinical trials of an improved anticoagulant is Food and Drug Administration licensure, which would permit clinical use of red cells after prolonged liquid storage. Shipment of blood into combat areas necessitates delays between drawing and infusion; the impact of these delays on the quality of red cells infused will be minimized through use of an improved anticoagulant-preservative solution

24. (U) Currently, red cell liquid storage in CPDA-1 anticoagulant-preservative is limited to 35 days and is not approved for component storage. Survivability of packed red cells (PC) stored in CPDA-1 for 35 days is marginally acceptable. In vitro studies of metabolism in red cells and platelets stored in modified CPD- adenine suggest that increased adenine and glucose in the preservative will improve survivability. Such improvements may allow extension of red cell storage time to 42 days or beyond. The Division of Blood Research, LAIR, will coordinate efforts with civilian and container-solution manufacturers in the execution of clinical trials of promising improved CPD-adenine formulations.

25. (U) 78 10 - 79 09. An improved CPD-adenine anticoagulant-preservative, CPDA-2, has been evaluated in vitro and shows promise of extending blood storage beyond 35 days. Human in vivo red cell survival studies are currently underway in an effort to extend blood storage to 42-56 days.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 004 CPD-Adenine Clinical Trials

The following investigations have been conducted under this work unit:

STUDY NO. 1 Platelet studies-CPDA-2

STUDY NO. 2 Red cell studies-CPDA-2

STUDY NOS. 1 and 2. Recent collaborative efforts with several laboratories were initiated and guided by the Division of Blood Research, LAIR, and culminated in the development and FDA approval of a new preservative, CPDA-1. CPDA-1 was a marked improvement over CPD and ACD, by extending shelf life 67% and improving the quality of preserved red cells. CPDA-1 preservative is not optimal and in vitro studies suggest that improved formulations may extend storage beyond 35 days, even for packed red cells. The preservative CPDA-2 has been selected as the best formulation of adenine and glucose. To establish human utility, and to obtain FDA approval, clinical trials for both red cells and platelets must be performed. Red cell collaborative studies have been established with Dr. E. Beutler, La Jolla, CA, and the American Red Cross Research Laboratory, Bethesda, MD, to document the efficiency of the preservative, CPDA-2, which has been determined to be the best formulation of adenine and additional glucose. Red cell and platelet protocols have been developed and approved for in-house studies.

BODY OF REPORT

WORK UNIT NO. 004 CPD-Adenine Clinical Trials

STUDY NO. 1 Platelet Studies CPDA-2

PROBLEM

Before FDA approval of a new preservative, it must be documented that the solution will not adversely effect any usable component of blood such as plasma proteins and platelets. Approval of CPDA-1 was delayed due to the lack of data concerning the effect of the preservative on platelets. CPDA-2, the new preservative developed in part by the Division of Blood Research to optimize the concentration of adenine and glucose for red cell storage, is now ready for clinical trials. Concurrent with red cell studies, platelet studies should be performed to insure the preservative is not injurious to this blood component.

RESULTS AND DISCUSSION OF RESULTS

Two protocols have been generated and submitted through command channels to the Human Subjects Research Review Board. The Surgeon General accepted the recommendation of the Board and approved the protocol. Recruitment of volunteers has begun. One protocol addresses recovery and survival of platelets stored in CPDA-2 and infused in normal volunteers. Data in our laboratory suggest this preservative may be beneficial for storing platelets. Therefore, prolonged storage (96 hours), as well as conventional storage (72 hours), is planned. Eight-hour preprocessing delays will also be studied. The second protocol addresses in vivo function; thrombocytopenic patients will be solicited to serve as volunteer subjects.

CONCLUSIONS

The protocols are designed to answer questions related to the storage of platelets in the presence of CPDA-2.

RECOMMENDATIONS

These studies should be implemented during FY 80 to assure the most cost-effective approach to preservative approval and acceptance.

PUBLICATIONS

None

CPD-Adenine Clinical Trials

STUDY NO. 2 Red cell studies-CPDA-2

PROBLEM

Recent collaborative effort by several laboratories was initiated and guided by the Division of Blood Research, LAIR, and culminated in the development and FDA approval of a new preservative, CPDA-1. This preservative was a marked improvement over CPD and ACD by extending shelf life from 21 days to 35 days and improving the quality of the red cells. CPDA-1 is not an optimal preservative particularly for packed red cells stored 35 days. Studies in this laboratory suggest improvements in the concentrations of adenine and additional glucose could result in greater than 35-day storage and improve the capability to store packed red cells. This approach has marked impact for military needs since prolonging the ability to store blood will improve logistical support for combat zone needs. The better the preservative, the more universal its acceptance. The use of a military oriented preservative by civilian blood banks will attempt to insure that military needs can be met from existing blood supplies.

RESULTS AND DISCUSSION OF RESULTS

A protocol has been generated to evaluate CPDA-2 in human clinical trials. Intramural studies are designed to evaluate 5 units of whole blood, 5 units of packed red cells (each at 35, 42, 49, and 56 days of storage) to determine the maximum acceptable length of storage. Packed red cells will be prepared from whole blood held for 8 hours prior to processing. This protocol has been developed in conjunction with Dr. E. Beutler, La Jolla, CA and the American Red Cross Research Laboratory. These laboratories will collaborate in the studies to provide extramural documentation for FDA approval. The protocol has been approved by The Surgeon General based upon the recommendation of the Human Subjects Research Board.

CONCLUSION

This study is designed to gather data for FDA approval of the new preservative, CPDA-2. When implemented it will establish the maximum storage capability provided by the preservative and is designed so the data can be used most efficiently in conjunction with collaborative laboratories.

RECOMMENDATIONS

The protocol should be implemented in FY 80.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6099	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 15	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	007 APC 505H
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code): (U) Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002600 Biology; 003500 Clin Medicine; 009800 Medical and Hosp Equip; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
75 07	CONT	DA			C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: Not applicable
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	0.8	12
FISCAL YEAR CURRENT 80	2.0	43

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: POC: DA

21. GENERAL USE
Foreign Literature Reviewed

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Rapid Screening Techniques; (U) Blood Preservation; (U) Temperature; (U) Blood Respiratory Function

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Testing of candidate blood preservatives at low tempratures (4 C) is a lengthy process due to low rate of change in critical parameters. The objective of this work unit is the development of a rapid screening method at elevated temperatures to reliably test efficacy of blood preservation solutions and techniques.

24. (U) Results show that the rates of change of functionally significant substances in blood stored at 4 C can be predicted from observations at 37 C. As rates of change are often 30-fold greater than at 4 C, observations at 37 C of the effects of experimental anticoagulant-nutrient preservatives on blood storage can be condensed to about 24 hours compared to 35 or so days required at 4 C. This approach to such screening will be extended to (a) optimize the composition of preservative media, (b) identify factors altering the collection and storage lesions, and (c) understand differences in individual donors with respect to blood storageability.

25. (U) 78 10 - 79 09 Oxygen transport function in red cells exposed to conditions simulating those during the first 20% of blood withdrawal from donors (high acidity, elevated temperature, excess CPD) is substantially and rapidly altered. Although 2,3-diphosphoglycerate is rapidly lost, oxygen affinity of red cells in this initial fraction of collected blood is paradoxically lower than normal. This behavior appears to be associated with an altered transmembrane pH and an increased intraerythrocyte acidity when these cells are returned to normal environment (pH ~ 7.4). Because the conditions under which red cells are collected modify the survival and quality of transfused stored blood, these observations will be expanded to provide more information regarding the effect of such conditions on 24-hour in vivo survivability and the functional quality of transfused red cells. Means of ameliorating this collection lesion will be investigated utilizing the techniques previously developed within this work unit.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 007 Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

Incubating erythrocytes in citrate-phosphate-dextrose (CPD) at 37 C produced rapid changes in transmembrane pH that are correlated with slowly developing changes observed in 4 C storage of red cells in CPD. These changes, which can produce an unusually low intraerythrocyte pH when cells are resuspended in plasma, cause a right shift in the hemoglobin oxygen affinity due to the Bohr effect. Similar changes are found with propranolol and ouabain. The changes in pH and oxygen affinity are reversible, presumably due to the relatively slow cation equilibration across the membrane. These alterations have been more pronounced in older high density cells compared to young cells, an effect perhaps related to a higher activity of the cation transport system in young cells. Monitoring the velocity of intracellular pH changes indirectly from the Bohr effect on oxygen affinity may be a useful in vitro test for predicting in vivo survivability of stored blood. The alterations in transmembrane pH are most prominent during the first 20% fraction of harvested blood cells, coinciding with alterations related to the collection lesion.

BODY OF REPORT

WORK UNIT NO. 007 — Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

PROBLEM

The supply and quality of liquid stored blood used for transfusion purposes in wounded soldiers can be seriously limited by the exigencies of warfare. Therefore, there is a unique military requirement for prolonging the present storage limit for preserved blood and improving the quality of this product. Most experiments designed to improve preserved liquid blood generally are performed at 4 C, presumably on the pragmatic ground that this temperature normally is used for liquid blood storage. For several reasons, however, this pervasive experimental approach may be undesirable. In the first place, the practice tends to perpetuate the notion that 4 C is the optimum temperature for blood storage; actually, this idea has not received extensive experimental inquiry. Furthermore, individual experiments at 4 C usually require prolonged periods of time to complete (up to 6 weeks or more). Because of the variability found in the blood of individual donors and the multiple permutations in storage conditions and preservation media that may require testing, satisfactory confidence in a new media or preservation idea can be slow in developing. Finally, phenomena important to the full understanding of the collection and storage lesions may be suppressed or imperfectly developed at 4 C. Therefore, the objective of this work unit is to devise accelerated means for evaluating blood preservatives and other variables affecting red cell storage in order (1) to economize on valuable technical resources, (2) to provide alternative theoretical approaches to understanding the deterioration of red cells during collection and storage, and (3) to develop a scientific basis for determining the optimal liquid storage temperature of blood.

RESULTS AND DISCUSSION OF RESULTS

Normal erythrocytes suspended in plasma at pH 7.4 and 37 C maintain an intraerythrocyte pH of approximately 7.2. Maintenance of this hydrogen ion gradient is explained by the Donnan effect on distribution of charged ions across membranes and expression of this latter effect is modified by the existence of active transport systems for cations that maintain concentration gradients of Na and K with metabolic energy. Perturbation of erythrocytes in equilibrium with the external environment, for instance by altering the external pH, disrupts this equilibrium and a new steady state is established by rapid adjustments of Cl^- and HCO_3^- as well as by the much slower cation transport processes. The H ion gradient will be modified by both of these adjustments.

Because the measurement of ionic activities inside cells is experimentally difficult, it is not surprising that much of the information on the functional and metabolic properties of erythrocytes (as well as other cells) is referred to conditions in the external environment. For instance, the oxygen affinity of hemoglobin in erythrocytes is conditioned by the intraerythrocyte pH, although the majority of such measurements are related to the pH of the external media. Thus, the transmembrane pH gradient and the factors that modify this gradient are important for understanding the functional and metabolic alterations that occur during blood storage.

In the present reporting period considerable effort has been devoted to observing the effects of 37 C incubation of erythrocyte in citrate-phosphate-dextrose (CPD) nutrient-preservative media on the alterations in transmembrane pH. The correlates of these effects with those observed at 4 C storage temperatures have also been studied. The intraerythrocyte pH has been measured directly with a capillary electrode using hemolysates of packed cells. Alternatively, intraerythrocyte pH has been inferred by measuring the oxygen affinity of hemoglobin.

When red cells are exposed to the low pH existing in CPD solutions, there is a prompt decrease in the intraerythrocyte pH. The first 20% portion of donor blood that is harvested may have an intraerythrocyte pH below 6.0, but this change is less than the pH change in the external media. Thus the pH gradient across the membrane actually reverses with a similar reversal in the membrane potential difference. When these cells are returned to a normal pH environment, they do not immediately assume the usual transmembrane pH pattern. Instead, depending on the time of incubation, large gradients are observed. Intracellular pH values as low as 6.8 have been observed when these incubated red cells are resuspended in a buffer at pH 7.4. As might be expected from the Bohr effect, the hemoglobin in these cells has an extremely low oxygen affinity even though minute quantities of organic phosphates are present. Older erythrocytes require less incubation time in CPD than do younger cells in order for this effect to occur.

Generally similar effects have been observed in blood storage experiments at 4 C. Although it is generally assumed that the loss of organic phosphate, particularly 2,3-diphosphoglycerate, is directly correlated with an increase in hemoglobin oxygen affinity, this does not appear to be true when cells are stored in CPD or CPD-adenine. In such cases, P_{50} may actually increase or remain unchanged initially, despite sizable losses of organic phosphates and 2,3 diphosphoglycerate. This effect, however, is associated with an increased transmembrane pH and lowered intraerythrocyte pH. Thus, it would appear that this

effect is similar to that seen with propranolol and ouabain, both of which lower potassium and increase hydrogen ion intracellularly. Both compounds likewise decrease the oxygen affinity of red cell hemoglobin; this decrease, also can be accounted for by the Bohr effect. Since transfused stored erythrocytes are known to require an extended period of time to restore intracellular potassium (and presumably pH) to normal levels, it is perhaps reasonable to suggest that active cation transport and transmembrane pH are major factors in modulating the oxygen transport function of stored blood. In this regard, we note that organic phosphates, including 2,3 diphosphoglycerate, do not have a marked affect on oxygen affinity when ionic strengths similar to those found in red cells are present. In fact, the difference between oxygen affinity of hemoglobin in normal red cells and in hemolysates free of organic phosphates can be accounted for almost entirely by the transmembrane pH effect (Bohr factor) and the effect of hemoglobin concentration.

CONCLUSIONS

An important functional attribute of red cells, the transmembrane pH, is significantly altered by collection and storage in CPD and CPD-adenine.

Changes in the transmembrane pH can be inferred from alterations in the cellular hemoglobin oxygen affinity; this approach offers technical advantages over alternative techniques because it is "non-invasive" and can be measured accurately and rapidly.

Transmembrane pH and cation transport rates of stored blood may be a potential means of predicting *in vivo* survivability of transfused erthrocytes.

RECOMMENDATIONS

The study should be continued in order to determine whether or not these findings can be exploited to improve the quality and storage capacity of erythrocytes for use in remote combat zones.

PUBLICATIONS

Publications stemming from this work unit were listed in the LAIR Annual Research Progress Report 1978, p 350.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6306	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	H.TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	009 APC 505H
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Metabolic Support Following Combat Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 008800 Life Support; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	79 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	6.4	161
	CURRENT 80	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Division of Surgery

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NAME: Scott, Rhonda L., CPT, MSC
NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Body Compositional Change; (U) Wound Healing; (U) Military Trauma; (U) Parenteral Nutrition; (U) Animal Model

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Combat injuries produce a uniquely severe form of stress to normal metabolism. Within days following significant combat injury, up to 21% of body muscle mass may be lost. Improved techniques of metabolic support may minimize this deleterious body compositional change, maximize wound healing, and reduce morbidity. The objective of these studies is to develop optimal post-traumatic metabolic support techniques and formulae to facilitate rapid return to duty.

24. (U) An in vitro perfused rodent hind limb, wounded to simulate combat injury, has been prepared. Using this preparation, we can study the effects of the post-traumatic hormonal milieu on normal and wounded muscle substrate utilization, and can design a post-traumatic regimen which can be deployed in field settings. It is planned to test the metabolic support formulae in higher animals and in traumatized man.

25. (U) 78 10 - 79 09 Carrageenan injections into the muscle of the rat hind limb result in a severe inflammatory response progressing to fibrosis that simulates histological and metabolic changes following combat injury. Marked alterations in amino acid release and glucose utilization have been shown. Somatomedin-C has been shown to markedly inhibit glucagon-stimulated gluconeogenesis, thus providing a potentially potent agent to modify the metabolic response to trauma. Intravenous substrate supply has been shown to markedly alter the plasma distribution of fatty acids and significantly impact on the diagnosis of essential fatty acid deficiency. This work unit has been terminated due to transfer of the principal investigator.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 009 Metabolic Support Following Combat Injury

The following investigation was conducted under this work unit:

STUDY NO. 1 Metabolic support following combat injury

STUDY NO. 1 A model has been developed in 180 to 200 g male rats which permits isolation of skeletal muscle metabolism apart from the remainder of the animal. As a consequence, the influence of neurohumoral control and substrate supply on skeletal muscle metabolism can be carefully controlled. Initial evaluation of the preparation involved determination of the presence of adequate perfusion and normal hormonal sensitivity.

Subsequently, an isolated perfused muscle wound model has been developed by using carrageenan injections into muscle. This preparation appears to be histologically and metabolically similar to a healing wound; thus, a setting has been created whereby a study of hormone substrate interrelationships in wound healing in combination with the effects on normal muscle can be made.

ADDENDUM As described in the last report from this work unit, tasks under the protocol "Studies in Combat Wound Healing" (Project No. 3S762772A814, WU 010; terminated 1 October 1977) designed by LTC Stuart Gourlay have been incorporated to prevent waste of substantial supplies. The previous studies demonstrating the effect of intravenous substrate supply on plasma concentration of essential fatty acids have been extended. These investigations have produced important insight into the diagnosis of essential fatty acid deficiency. Work unit terminated due to transfer of the principal investigator.

BODY OF REPORT

WORK UNIT NO.	009	Metabolic Support Following Combat Injury
STUDY NO.	1	Metabolic support following combat injury

PROBLEM

Combat injuries present the most pronounced example of stress metabolism. The response to this type of trauma is one of extreme catabolism which often results in body compositional losses of over 1 kg (2.2 lb) daily. These compositional changes reflect losses of stored carbohydrate (glycogen), triglycerides (subcutaneous fat), and muscle mass, which are rapidly utilized for energy by the altered post-trauma metabolism.

Body fat is considered an efficient form of stored calories, and its utilization for this end during periods of high caloric requirements, is not generally considered detrimental. However, muscle is an important structural element and, as a consequence, relatively small losses of muscle mass result in significant alterations in total body function. With severe injury, the loss of body nitrogen is accelerated from 3 to 5 g per day (the loss during times of simple starvation) to a range of 15 to 30 g per day for several days. The loss of nitrogen is reflected by loss of muscle mass; therefore, with severe injury, the loss of nitrogen represents 450 to 900 g loss of muscle mass. Caloric supply during this period will spare body fat but is thought to have little effect on loss of body nitrogen. Loss of limb muscle mass leads to marked weakness and impaired mobility. Loss of intercostal and diaphragmatic muscle mass leads to inability to clear bronchial secretions and may result in pneumonia and death.

The interrelationship between the general biochemistry of injury and convalescence and the local changes of wound healing has not been investigated in detail. It is known that early wound healing will occur during a period of negative energy balance, i.e., general catabolism. There is clearly a high biological priority of the wound in the early days and weeks after injury. This favored biological priority, however, is transient. One to two weeks following trauma, there is of necessity a prolonged phase of protein synthesis and lipogenesis to restore tissue mass to normal. At this point, if intake of foodstuffs can be resumed, wound healing moves normally toward completion. If, however, there is prolonged starvation and continued catabolism, the wound must begin to compete with other tissues for substrate, and wound healing begins to suffer severely.

The objective of this investigation is to develop a specific therapeutic regimen for post-traumatic metabolic support that will facilitate the rate of wound healing while minimizing body compositional changes. The steps necessary to accomplish this objective are as follows: (1) investigation of the effect of the post-trauma hormonal milieu on substrate utilization by normal muscle; (2) investigation of substrate utilization by wounded muscle and the alterations in this utilization by the post-trauma hormonal abnormalities; (3) design of a post-trauma therapeutic regimen based on steps 1 and 2 that will maximize the rate of wound healing while minimizing muscle compositional changes; (4) investigation of this formula in traumatized dogs and nonhuman primates; (5) investigation of this formula in traumatized man; and (6) modification of this formula as necessary for feasibility of use under combat conditions.

RESULTS AND DISCUSSION OF RESULTS

The first phase of this study involved development of the experimental model to study muscle metabolism in situ. The isolated perfused rat hindquarter technique was evaluated for viability and effect of insulin on glucose clearance.

The second phase of this study involved the development of an isolated perfused wounded rodent hindlimb model that would simulate both metabolically and histologically a healing combat injury. The objective was to replace as much muscle mass as possible with the wound without interfering appreciably with blood flow. Three types of experimental wounds were evaluated as to their effect on animal weight change following wounding and gross anatomical replacement of muscle mass with fibrous tissue. All three techniques produced an obvious inflammatory reaction. Although the Ivalon sponge and stainless steel wound chamber techniques produced a localized response, it was technically difficult to place sufficient numbers of these devices in the hindlimb because placement of large numbers of these devices required removal of muscle mass to the point of vascular impairment. Carageenan injections, however, allowed uniform distribution of the wounding agent without vascular injury.

Since substrate utilization in any perfused system is dependent to a significant degree upon perfusate flow rate, it was necessary to demonstrate that the wound did not interfere with flow rate. Evidence indicates that the wound did not interfere with the flow rate (1% carrageenan: 9.75±0.2 ($\bar{X}$±SEM) versus control 9.94±0.1 ml/min and 1.5% carrageenan 10.7±0.2 versus control 9.4±0.25 ml/min). No edema or hematoma formation occurred during perfusion.

Subsequent studies have evaluated substrate utilization by this isolated perfused rodent wounded hindlimb model. These studies revealed a fourfold increase in glucose clearance and a threefold increase in lactate production in the perfused wounded hindlimb. Amino acid analysis revealed significant changes in the release of leucine (fourfold), valine (twofold), methionine (two and one-half fold), and the uptake of serine (twofold).

CONCLUSIONS

Preliminary data indicate the feasibility of a perfused wound preparation that has the characteristics of (1) replacement of a major portion of the hindlimb with a metabolically active wound, (2) no interference with perfusate flow rate, and (3) a significant injury to the animal. Biochemical analyses of substrate utilization indicate that this model is representative of an active wound. This model should facilitate other investigations in the study of hormone-substrate interrelationships in the healing wound.

RECOMMENDATIONS

Work unit terminated due to transfer of principal investigator.

PUBLICATIONS

None

ADDENDUM

PROBLEM

Work Unit No. 010, Project No. 3S762772A814, "Studies in Combat Wound Healing," under the direction of LTC Stuart Gourlay was terminated at the end of FY 76 because of the departure of the principal investigator. These studies (to investigate the requirements for essential fatty acids in combat injured individuals) were then incorporated into our ongoing research to prevent waste of substantial supplies.

The need for essential fatty acids (EFA) in animals has been recognized for a long time; without EFA, a syndrome characterized by caudal necrosis of the tail, scaly skin lesions, impaired growth, renal degeneration, and premature death develops. The occurrence of essential fatty acid deficiency (EFAD) in humans has been documented only recently, and the only clearly proven effect of EFAD in adult humans so far is a characteristic scaly dermatopathy. Biochemical serum fatty acid patterns of EFAD predate the appearance of cutaneous lesions, and their onset are markedly accelerated by severe trauma. There is evidence of

a significant delay in human wound healing with EFAD, but no studies to define the role of EFA in wound healing have been undertaken, although the importance of EFA in maintenance of plasma membrane integrity suggests that EFAD may interfere with fibroplasia. Delivery of adequate EFA to prevent EFAD in combat casualties in forward hospitals may be necessary if EFAD significantly retards the rate of healing.

RESULTS AND DISCUSSION OF RESULTS

Two specific experiments were undertaken: (1) evaluation of the effect of essential fatty acid deficiency on membrane formation and ability to metabolize injected medications; (2) effect of the intravenous supply of various substrates on EFA mobilization and utilization.

Experiment 1. Essential fatty acid deficiency markedly alters the biochemical composition of all membranes. It has been postulated that these biochemical alterations would interfere with membrane function and possibly membrane synthesis. This should become extremely important for membrane repair following tissue injury and may account for the delayed wound healing found in EFAD animals. Initial experiments to evaluate the role of EFA in membrane synthesis involved the administration of phenobarbital (which stimulates microsomal membrane synthesis) to EFAD and rat chow fed animals. It was found that the EFAD animals did not have the normal smooth endoplasmic reticulum proliferation in response to phenobarbital injection. As a consequence, studies are underway to evaluate the effect of EFAD on mixed function oxidoses. These studies should give an indication of the role of EFA supply on an animal's ability to metabolize various medications.

Experiment 2. It has been suggested that an alteration in fat-free intravenous substrate supply can markedly change the requirements for essential fatty acids. As a consequence, EFAD, EFAD + linoleic acid supplement to meet requirement, and chow-fed control animals were placed on fat-free intravenous regimens. Data seem to indicate a marked difference in EFA metabolism; this difference probably occurs at the tissue level. That possibility is being investigated.

CONCLUSIONS

Amino acid solution infusion (protein sparing) does not prevent or relieve pre-existing EFAD. Although administration of amino acid solution maintains a normal fatty acid pattern, the total body content of linoleic acid is greatly depleted. Total parenteral nutrition apparently produces artificial biochemical manifestations of EFAD due to increased synthesis of nonessential fatty acids.

RECOMMENDATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6077	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 15	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	012 APC 505H
b. CONTRIBUTING				
c. xxxxxxxxx	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support; 016200 Stress Physiology; 009800 Medical and Hospital Equipment

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 11	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	0.3	14
FISCAL YEAR CURRENT 80	0.1	19

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U)Combat Surgery; (U)Trauma; (U)Wet-Lung Syndrome; (U)Left Ventricular Function; (U)Oxyhemoglobin Disc; (U)Artificial Blood; (U)Combat Anesthesia

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Newly developed artificial blood substitutes and whole blood stored using new techniques must be physiologically evaluated to insure their ability to support tissue function. The objective of this work unit is to develop and use an appropriate subprimate model which will permit precise measurements of ventricular hemodynamic and metabolic function. This model has been used to investigate the importance of different anesthetic agents under conditions of combat stress, and to test the effectiveness of newly developed blood substitutes and blood having altered oxyhemoglobin dissociation characteristics.

24. (U) A perfused in situ swine heart model using total and right heart bypass with control of heart rate, blood pressure, and left heart loading pressure has been employed. Left heart performance and myocardial oxygen transport dynamics will be assessed to determine the ability of newly developed blood substitutes and anesthetic agents to support normal tissue function under combat stress.

25. (U) 78 10 - 79 09 A cardiovascular investigational laboratory is functioning for accurate measurements of stroke volume, dp/dt, ejection fraction, myocardial metabolism and coronary flow distribution. Investigations using hemoglobins with altered affinity for oxygen revealed decreased ventricular function and oxygen extraction with a decrease in P_{50}. Initial studies with stroma-free hemoglobin solutions used in this model revealed adequate support of myocardial function at a hematocrit of 5% with stroma-free hemoglobin solution but inadequate support using albumin solutions. Evaluation of various anesthetic techniques during hypoxia have revealed marked myocardial depression with halothane anesthesia that is accompanied by an increase in heart oxygen demand. Current investigations are focusing on blood substitutes and anesthetic agents and the large amount of accumulated data is continuing to be analyzed.

*Available to contractors upon originator's approval

DD FORM 1498, MAR 68 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 012 Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

The following investigations are being conducted under this work unit:

STUDY NO. 2 The effect of variation in the oxyhemoglobin dissociation curve on left ventricular function in swine

STUDY NO. 3 Anesthetic agents and their effect on left ventricular function during normoxia and hypoxia

STUDY NO. 4 The effect of stroma-free hemoglobin solution on myocardial function in a non-shock, sub-total exchange model

STUDY NO. 2. The relationships between preservation of myocardial performance and the oxyhemoglobin dissociation curve of priming solutions have been investigated in the isolated swine heart preparation described previously. These studies have been designed to determine whether or not the P_{50} of resuscitation fluids, including whole blood, is a significant determinant of recovery from hemorrhagic shock secondary to massive combat wounds. Animal studies have been completed indicating that variations in P_{50} have a significant effect on left ventricular function at normal oxygen tensions and hemoglobin concentrations. Further studies examining the role of P_{50} variation during anemia are presently under evaluation and preliminary analysis shows that anemia may not heighten the effects of change in P_{50} on left ventricular function. Further work is underway to determine whether or not the oxyhemoglobin dissociation curve has an important part in determining myocardial performance during hypoxia and limited coronary artery blood flow.

STUDY NO. 3. The myocardial effects of the major anesthetic agents have been studied in our swine heart model in an attempt to evaluate these agents under conditions analogous to combat-induced stress. Our initial studies examined the myocardial consequences of morphine and halothane in animals subjected to hypoxia at a level of 40 mm Hg. This initial study has essentially been completed with the finding that halothane anesthesia does indeed depress myocardial performance, but does it while decreasing myocardial oxygen consumption as well. During periods of hypoxia, the decrease in myocardial function is further exacerbated and, unfortunately, this decrease in function is not accompanied by a

decrease in myocardial oxygen consumption, thereby leading to a disadvantageous myocardial work per unit of oxygen consumption balance. Our investigations in respect to morphine anesthesia revealed that this agent had no effect on depressing myocardial function during normoxia and that it decreased myocardial performance only to a moderate degree during hypoxia. Further studies are being continued to help determine the mechanism of this decreased performance under combat stress.

STUDY NO. 4. Work has continued to progress in the evaluation of current and proposed stroma-free hemoglobin solutions on myocardial performance. In initial studies with the in situ swine heart model, we evaluated a subtotal exchange transfusion comparing the stroma-free hemoglobin solution prepared by crystallization with a 7% bovine-albumin solution. These studies showed that, while myocardial performance was decreased by approximately 50% with stroma-free hemoglobin solution, the animals were able to maintain this level of cardiac performance whereas animals exchanged with the albumin solution were unable to sustain any degree of myocardial work. Further studies are in progress in an attempt to determine whether or not this advantageous situation with hemoglobin solution will occur at hematocrit levels above 5 volumes percent.

BODY OF REPORT

WORK UNIT NO. 012 — Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

STUDY NO. 2 — The effect of variation in the oxy-hemoglobin dissociation curve on left ventricular function in swine

PROBLEM

Recently, with the understanding that the oxyhemoglobin dissociation curve is affected by concentrations of 2,3-DPG and that stored blood has a low 2,3-DPG level, there has been concern that massive transfusions with blood which has been stored for prolonged periods may have a detrimental effect on oxygen delivery to critical tissues. Myocardial function is intimately tied to adequate oxygen transport which, if less than optimal, may depress heart performance in the combat-injured soldier. Some studies have suggested that there is a relationship between P_{50} and left ventricular performance. If an adequate P_{50} is crucial to preserving heart performance during periods of combat injury, then aged blood with a low P_{50} and low 2,3-DPG may have limited usefulness, and fresh blood or blood with enriched 2,3-DPG must be made available. If P_{50} is not a major determinant of left ventricular function, aged blood could be employed, especially during combat situations which would require massive transfusions and maximal utilization of blood bank resources.

RESULTS AND DISCUSSION OF RESULTS

Our in situ perfused swine heart model has been used for this study. As previously outlined, left ventricular function and metabolic responses have been directly evaluated.

This study continues with evaluation of myocardial function following exchange transfusions with blood having various P_{50} characteristics and hematocrit levels. As reported last year, our initial study with this preparation examining the situation at a normal hematocrit showed that the left ventricular performance is affected in an adverse fashion when animals are subjected to blood having a lowered P_{50}. This change in performance was accompanied by documented and statistically significant changes in the P_{50}, n-value, and coronary sinus blood gas values for the animals. The group of animals subjected to exchange with high P_{50} blood had preservation of myocardial performance but did not show an improved or super performance with the blood having an elevated P_{50} value. An additional phase of this study is in progress examining the effect of altered P_{50} in the left ventricular function in an animal

exchanged with blood at a lowered hematocrit level. Most of these animal studies have been completed and the preliminary analysis has failed to show as dramatic an effect of P_{50} on left ventricular function as might have been expected from the previous study performed at a normal hematocrit level.

CONCLUSIONS

Our basic conclusion continues to be as was reported in our previous year's report, that P_{50} is indeed an important determinant of left ventricular function as evidenced by our first completed study. The question of its clinical importance remains to be answered and further elucidated by studies which will examine the situation in animals stressed at a lower hematocrit level, and in situations of decreased oxygen tension.

RECOMMENDATIONS

The findings in this study have helped to answer the question of how vital a role P_{50} changes play in myocardial performance; however, additional continuing work is obviously needed to weigh adequately this role in a clinical situation analogous to that experienced by soldiers on the combat field. The problem of evaluating the role of P_{50} in myocardial performance is being assessed with the present in situ swine model and with possible expansion to a less expensive small animal model. Recommendations at this time include the continued publication of animal experiments previously performed under this work unit as well as a continued work examining this question in additional animal models. Some of this work will be accomplished in the Letterman Army Institute of Research facility; however, much will also be accomplished in extramural laboratories with which the principal investigator is presently associated. We hope that the material from all of these studies will provide valuable information for the Army's attempt to determine the proper significance of preserving 2,3-DPG concentration levels in blood for transfusion in combat situations.

PUBLICATIONS

1. MOORES, W.Y., D.C. WILLFORD, J.D. CRUM, J.R. NEVILLE, R.B. WEISKOPF, and W.P. DEMBITSKY. Alteration of myocardial function resulting from changes in hemoglobin oxygen affinity. (Abstract) Circulation 58(Suppl II):225, 1978

2. MISBACH, G.A., S.A. GLANTZ, J.V. TYBERT, and W.Y. MOORES. Improved compliance and ventricular function curves after open-heart surgery: role of the pericardium. Surg Forum 29:251-253, 1978

3. HEYDORN, W.H., W.Y. MOORES, J. MACK, and W.P. DEMBITSKY. The importance of hyperkalemia in a cold perfusion solution: a correlative study examining myocardial function, metabolism, tissue gases and substrates. Ann Thorac Surg 28:281-289, 1979

STUDY NO. 3 Anesthetic agents and their effect on left ventricular function during normoxia and hypoxia

PROBLEM

The effects of anesthetic agents on myocardial function have been well worked out for the normal situation encountered in civilian operating room practice where the patient is at an optimum oxygenation level. Unfortunately, during combat situations patients may have to be induced during conditions of physiologic stress as exemplified by a decreased oxygen tension. The ultimate survival of these patients is closely connected with their myocardial performance and safe anesthesia would require optimization of this performance even during conditions of hypoxia. This information becomes crucial if the field anesthesiologist is going to select the optimal available anesthetic agent during these combat stress situations. This particular problem has been addressed primarily in a separate work unit (Work Unit No. 008) under the direction of a principal investigator in the anesthesia area; however, during the last year work in this field has been conducted under this work unit and is reported here.

RESULTS AND DISCUSSION OF RESULTS

As in the previous studies, the perfused swine heart model has again been used and animal studies examining the response of halothane, morphine, and infiltration anesthetic regimens have been conducted. The technique for accurately measuring anesthetic concentrations with a mass spectrometer and the technique for accurately adjusting the animal's oxygen tension to a level of 40 torr have been perfected. With these technical refinements, it has been possible to complete the evaluation of a series of animals at a normoxia and at the hypoxic level of 40 torr. The initial results from this study have shown that halothane, as expected, drops myocardial performance even during normoxia and that this drop in performance is accompanied by a drop in myocardial oxygen consumption as well. The new finding during hypoxia was that halothane anesthesia not only drops myocardial performance significantly more during hypoxia, but that this drop in performance is not accompanied by a corresponding drop in oxygen consumption. The experiments performed with morphine anesthesia substantiated that, under conditions of normoxia, morphine has no appreciable depressive effects on myocardial performance and that its depressive effects

during hypoxia are relatively less than with halothane (approximately 25% versus 66%) and that this depression in function is not accompanied by an increase in myocardial oxygen consumption. The results from the animals examined under infiltration anesthesia were remarkably similar to those with the animals examined under morphine anesthesia. Analysis of these runs has continued in an attempt to determine the mechanism for this change in myocardial performance seen with halothane. Some of our initial analyses seem to indicate that the depression in myocardial performance with halothane may be due to a change in ventricular compliance rather than due to a change in myocardial contractility.

CONCLUSIONS

Our basic conclusion at this point is that halothane may be an appropriate anesthetic agent to use during normoxic conditions since its depression in myocardial performance is accompanied by a decrease in myocardial oxygen consumption so that the amount of oxygen consumed per unit of cardiac work done is not increased in a disadvantageous fashion that can result in ultimate ischemic damage to the myocardium. Our findings during hypoxia seem to indicate that halothane is not a good choice for administering anesthesia since the depression of function is even further enhanced and that this depression is accompanied by an increased O_2 consumption, thereby subjecting the myocardium to a greater chance of ischemic damage. Additional work is needed to examine the anesthetic agents during periods of hypoxia and other situations of deranged physiology such as hypertension and anemia that are encountered in a combat injury situation.

RECOMMENDATIONS

The initial series of experiments has been completed; however, the question of an appropriate choice of an anesthetic agent during situations of combat stress needs to be answered by additional studies examining these anesthetic agents during anemia and hypotension in this controlled swine heart model. Some technique modifications will probably be required to answer the question completely as to whether the depression in myocardial performance with halothane is indeed due to a direct depression myocardial contractility or due to a change in ventricular compliance.

PUBLICATIONS

1. WEISKOPF, R.B., W.Y. MOORES, K.K. RIORDAN, M.I. TOWNSLEY, J.D. CRUM, D.C. WILLFORD, and W.P. DEMBITSKY. Left ventricular dynamics: a comparison of morphine and halothane during normoxia. Proceedings of the Annual Meeting of the American Society of Anesthesiologists, 1978, pp. 321-322

2. MOORES, W.Y., R.B. WEISKOPF, W.P. DEMBITSKY, and J.R. UTLEY. Comparative effects of halothane and morphine anesthesia on myocardial function and metabolism during cyanosis in swine. Surg Forum 30:221-223, 1979

STUDY NO. 4 — The effect of stroma-free hemoglobin solution on myocardial function in a non-shock, sub-total exchange model

PROBLEM

Resuscitation of the combat injured soldier may require the use of various artificial blood substitutes as well as whole blood, and these solutions must be adequately evaluated in terms of their effects on myocardial function. Several studies examining stroma-free hemoglobin solutions have been accomplished in a shock model; however, it is appropriate to examine the effects of these resuscitation techniques in an animal model that is designed so that we can evaluate myocardial function in a non-shock situation as might be encountered during recovery and convalescence from a combat injury. This study should help to prepare the surgeon practicing in the combat situation to determine if the casualties should be transfused with hemoglobin solution or an artificial blood substitute that carries oxygen, or if a non-oxygen carrying blood substitute, such as albumin solution, would be adequate and appropriate.

RESULTS AND DISCUSSION OF RESULTS

During the last year, the in situ perfused swine heart model has been used to evaluate effects of an exchange transfusion of stroma-free hemoglobin solution on left ventricular function. The standard parameters of myocardial performance (stroke volume, etc.) have been examined under conditions of controlled pre-load, after-load, and rate, and an index of myocardial metabolism and oxygen utilization has been used as well. These studies have been done with hemoglobin solution that has been exchanged in a pig animal model so that the subsequent hematocrit was at 5 volumes percent. An initial series of experiments comparing stroma-free hemoglobin solution with albumin solution at a hematocrit level of 5 percent has revealed that animals transfused with the stroma-free hemoglobin solution were able to maintain a work performance at approximately 50% of their control value and that they were able to sustain this level of work performance for the standard work trial period. The animals exchanged with albumin solution to a similar hematocrit level were initially able to support a level of cardiac performance; however, within minutes of the work trial period these animals were no longer able to perform any useful cardiac work. Those animals perfused with stroma-free hemoglobin solution did show

signs of inadequate oxygen delivery with high lactate levels; however, the ability of the hearts to work with the stroma-free hemoglobin solution appeared to be fairly conclusive.

CONCLUSIONS

Following this initial set of experiments, we have concluded that stroma-free hemoglobin solution seems to hold some promise in terms of supporting useful cardiac work under conditions of severe anemia. Support of cardiac function occurred even though this present form of hemoglobin solution has a depressed P_{50} characteristic with a left-shifted oxyhemoglobin association curve. The finding of significant lactate production during perfusion with hemoglobin solution has led us to the conclusion that continued work needs to be done in regards to improving our solution so that cardiac performance can be maintained without the evidence for anaerobic metabolism.

RECOMMENDATIONS

This initial work seems to be encouraging in regards to the potential for stroma-free hemoglobin solution to be an appropriate blood substitute. However, additional work is necessary in defining its role primarily in those situations where the hematocrit level is not as severely depressed. This information is needed if the field surgeon is going to make a reliable decision regarding the use of stroma-free hemoglobin solution in those patients not requiring complete replacement of their blood volume with an artificial blood substitute. The data from the initial experiments continue to be analyzed and prepared for publication and subsequent experiments examining the role of stroma-free hemoglobin solution under conditions of less severe anemia are currently in progress.

PUBLICATIONS

1. MOORES, W.Y., F. DeVENUTO, W.H. HEYDORN, R.B. WEISKOPF, M. BAYSINGER, and J.R. UTLEY. Extending the limits of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution. J Thorac Cardiovasc Surg, in press.

2. MOORES, W.Y., F. DeVENUTO, W.H. HEYDORN, R.B. WEISKOPF, M. BAYSINGER, and J.P. HANNON. Improved porcine myocardial performance during severe anemia using a stroma-free hemoglobin solution. Fed Proc, in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6100	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	013 APC 505H
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS 114I			

11. TITLE (Precede with Security Classification Code)

(U) Effect of Blood-Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 012900 Physiology; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 07	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	1.0	35
FISCAL YEAR CURRENT 80	2.0	43

19. RESPONSIBLE DOD ORGANIZATION

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NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Literature Reviewed

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Resuscitation Solutions; (U) Experimental Hemorrhagic Shock; (U) Trauma; (U) Blood-gas Transport

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Blood oxygen transport function is critical for delivery of oxygen to tissues after military trauma, particularly during conditions of hypovolemia and hypoxemia. The objectives of this study are: the study of hemoglobin-oxygen affinity after trauma and hemorrhage, continued evaluation of a simple, reliable technique to measure affinity, and the search for agents to manipulate affinity and their testing in shocked and hemorrhaged animals.

24. (U) The approach to this problem incorporates three areas: (a) design and improvement of techniques and equipment for performing unusual or difficult biomedical measurements related to oxygen transport function, (b) theoretical analysis of the oxygen transport function of blood and related physiologic systems, and (c) use of specialized animal preparations such as the swine heart by-pass model (see Accession No. DAOE 6077, work unit 814-012), to test and evaluate relevant questions regarding the implications of hemoglobin-oxygen affinity to tissue oxygen transport in the combat wounded soldier.

25. (U) 7810-7909. Further work has confirmed the limitations of current unmodified formulations of stroma-free hemoglobin as a blood substitute. An intensified interdisciplinary effort to correct these deficiencies has been formulated. A rat hemorragic shock model, using spontaneous recovery from graded hypovolemia is presently being used to test effects of altered hemoglobin oxygen affinity on recovery from shock. No consistent differences have been observed in resting oxygen consumption ($\dot{V}O_2$) of control rats (no hemorrhage) with markedly different hemoglobin-oxygen affinity values. Hemorrhaged animals with normal oxygen affinity display an immediate profound reduction in $\dot{V}O_2$ with slow spontaneous recovery. Further tests of the effects of increased oxygen-affinity in hemorrhaged animals is currently underway using this model.

*Available to contractors upon principal investigator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3S76277A814	Military Trauma and Resuscitation
WORK UNIT NO.	013	Effect of Blood Oxygen Affinity during Experimental Hemorrhagic Shock and Hypoxemia

A rat model has been developed to evaluate the effect of hemoglobin-oxygen affinity on tissue oxygen transport and mortality with graded hypovolemia. With this model, no difference in the recovery from hypovolemia was observed in rats with large differences in oxygen affinity. No recovery was observed in this model when a 50% blood volume loss was produced; 50% of the rats with a 40% blood volume loss recovered. In vitro experiments confirm that mixtures of stroma free hemoglobin and whole blood release oxygen mainly from erythrocytes at normal oxygen tensions; however, rats treated with cyanate salts, which significantly lower P_{50} of erythrocyte hemoglobin, gain weight and react to stress normally. Life-span also appears to be normal. Consequently, efficacy of stroma free hemoglobin in some applications may depend more on improving intravascular retention than on modifying the oxygen off-loading properties.

BODY OF REPORT

WORK UNIT NO. 013 Effect of Blood-Oxygen Affinity during Experimental Hemorrhage Shock and Hypoxemia

PROBLEM

A continuous supply of oxygen is a major requirement for maintenance of vital functions in man and animals. Compared to food and/or water deprivation, which may be tolerated in man for prolonged periods, oxygen deprivation can be fatal in minutes. Although some tissues withstand the effects of oxygen deprivation better than others, the body as a whole tolerates a deficit oxygen economy only to the extent that it can activate a variety of compensatory mechanisms designed to sustain the minimum metabolic needs of the tissues during emergencies. Trauma associated with bleeding produces an immediate threat to the oxygen economy of the body, and the effectiveness of compensatory adjustments designed to cope with this situation is a decisive factor in the clinical consequences of such trauma. Apart from the potentially lethal effects of oxygen deprivation, tissue hypoxic episodes can lead to permanent functional damage and prolong recovery from the basic injury.

The occurrence of trauma and hemorrhage in a remote and hostile battlefield setting presents several unique problems not ordinarily encountered during peacetime emergencies. Medical assistance may be delayed by the exigencies of warfare, thus extending the stress upon compensatory reserves. Fluid replacement therapy, moreover, is often compromised by the unsatisfactory quality of blood that has been transported and stored for an extended period of time before becoming available in forward areas. A number of factors are thought to contribute to lowering the quality of stored blood, including high potassium and citrate content, microaggregate formation, high acidity and viscosity, as well as increased oxygen affinity. It is generally recognized that fresh blood is a more successful resuscitation fluid than is older, stored blood, particularly for massive transfusions, but the specific reasons for this have not been well documented. The present studies have been designed to explore some of these problems with the aim of finding more effective therapies for hypoperfusion states. In addition, the use of a blood substitute, such as stroma-free hemoglobin, requires an understanding of tissue oxygen transport mechanisms in order to facilitate the optimization of such substitutes for combat casualty care.

Thus, there are unique circumstances justifying the military need to understand the complex compensatory mechanisms that sustain the oxygen economy of the body. The objective of this work unit is to evaluate

the oxygen transport function of blood and resuscitative solutions, particularly with reference to the potential role of hemoglobin-oxygen affinity in modifying compensatory reserves of combat personnel during trauma and environmental stress.

RESULTS AND DISCUSSION OF RESULTS

Considerable effort has been spent during the current reporting period in attempting to test a number of small animal models that would be useful for studying the effect of pharmacologic and physiologic manipulations on hypovolemia and hypoxemia. Because of the confounding effect of surgery, anesthetic agents, and length of the hypovolemic episode on the various models that often have been used, we sought a model that would minimize these factors. In addition it appears desirable for the present purposes to study a model displaying spontaneous recovery (no transfusion) rather than the more common Wiggers' model in which blood is returned after a varying period of low blood pressure or when "take-back" reaches a certain percentage of the removed blood volume. Spontaneous recovery may be more appropriate for investigating hypovolemia in combat casualties when it is desirable to focus on the normal compensatory mechanisms that come into play during such episodes. When evacuation of the combat-injured is delayed and/or resuscitative fluids are unavailable, a drug or "cocktail" that favorably influences these normal homeostatic mechanisms could be lifesaving.

Sprague-Dawley rats of both sexes and weighing approximately 300-400 grams have been used. A light methoxyflurane anesthesia is induced prior to blood withdrawal from the heart with syringe and needle. Blood volume is estimated (7.5% of body weight). When small amounts of blood are withdrawn, the animal recovers quickly (a few minutes). Using this technique for withdrawing larger amounts of blood (up to 50% of blood volume), we found a consistent pattern between mortality, hemorrhage volume, and oxygen consumption in the rats (approximately 75) that have been tested. This pattern was similar in rats with wide differences in oxygen affinity,i.e., P_{50} between 20 and 40, (high affinities were induced by including a cyanate salt in the drinking water for periods of days or weeks). Withdrawal of greater than 50% of the blood volume has been uniformly lethal. Forty percent withdrawal was associated with a 50% mortality, and 30% withdrawal gave approximately 15% mortality. No deaths from hemorrhage of 25% or less were observed in this model. Oxygen consumption ($\dot{V}_{O_2}$) was measured continuously in some of these animals starting immediately after blood withdrawal and continuing for 20 to 25 minutes. Normal steady-state $\dot{V}_{O_2}$ in unanesthesized rats resting quietly in the measuring chamber is approximately 12-15 ml/min/kg. Unhemorrhaged but lightly anesthesized animals have a $\dot{V}_{O_2}$ similiar to resting unanesthesized

rats. For the first 5-10 minutes after hemorrhage, $\dot{V}_{O_2}$ dropped as low as 2 or 3 ml/min/kg; it gradually increased to normal during the next 15-20 minutes. No indication of an oxygen-debt payback has been observed; core temperatures remained near 37 C throughout this period. The initial drop in $\dot{V}_{O_2}$ appears related to the amount of blood withdrawn.

Although further studies are needed to characterize fully this "shock" model, the failure to observe any observable difference in the response of high versus low oxygen affinity rats appears to be based on the fact that homeostatic mechanisms are largely intact in this purposely simple preparation. Fluid recruitment from extravascular spaces, local autoregulation and generalized autonomic nervous and sympathomimetic processes are probably all playing a protective role in combating what is perhaps a fairly pure hypovolemic stress. These compensatory mechanisms can apparently override an isolated aberration such as represented by increased oxygen affinity. This may not be true in the more biochemically complex settings represented by prolonged or "irreversible" hypoperfusion.

To the extent that a blood substitute, such as stroma-free hemoglobin would be used for situations in which homeostatic mechanisms were intact, these results indicate that efforts to increase P_{50} in stroma-free hemoglobin may be unnecessary. Thus, the major effort to improve this solution perhaps should be directed at the intravascular retention problem.

CONCLUSIONS

A simple experimental rat model useful for studying the pharmacologic and physiologic responses during the initial phases of hypovolemia has been developed. The advantage of this model resides chiefly in the retention in the preparation of normal homeostatic mechanisms.

Hemoglobin-oxygen affinity does not significantly affect mortality or $\dot{V}_{O_2}$ during hypovolemia when homeostatic mechanisms are intact.

Efforts to improve stroma free hemoglobin should be directed initially at improving intravascular retention rather than P_{50} because, for some applications, of this blood substitute the P_{50} may not be a crucial factor.

RECOMMENDATIONS

The developed model should be characterized in more physiologic detail. The model can be applied to studying candidate pharmacologic agents for

combating the effects of hypovolemia and poisonous chemical agents either separately or in combination.

PUBLICATIONS

DEVENUTO, F., H.I. FRIEDMAN, J.R. NEVILLE, C.C. PECK. Appraisal of hemoglobin solution as a blood substitute. Surg Gynecol Obst 149:417-436, 1979.

Other publications based upon research accomplished under this work unit were listed in the LAIR Annual Research Progress Report 1978, pp 385-386.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6108	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 15	D. CHANGE		U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. [redacted]	62772A	3S162772A814	00	015 APC 505H
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Animal Models for Surgical Repair of Musculoskeletal Structures

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology; 003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 79	0.9	26
CURRENT FISCAL YEAR 80	1.0	27

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ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Surgical Repair; (U) Extensor Tendon; (U) Nerve; (U) Muscle Transplantation; (U) Trauma; (U) Nerve Graft; (U) Microsurgical Technique

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Extremity injuries in military personnel are extremely costly. To minimize the resulting lost duty days, permanent disability, and the expenditure of medical resources, efforts are being made to improve current surgical therapeutic injuries in order to return personnel to duty with maximum function in the minimum time.

24. (U) Segmental damage to the ulnar nerve in cats was repaired either by interfascicular graft or an epineurial technique under tension. Critical evaluation of the neurorrhaphies were made 6 months after repair. To determine the rate and morphometric pattern of axon regrowth across an anastomosis, ulnar nerves of 6 rhesus monkeys were severed, repaired, and then biopsied at either 1, 2, 3, 4, 5, or 6 weeks. To determine the effects of different nerve deficits, 16 cynomologus monkeys underwent resection of 0, 1, 2, or 3 cm of each ulnar nerve and repair epineurially with tension or interfascicularly with sural nerve grafts.

25. (U) 78 10 - 79 09 There was no statistical difference between the 2 repair techniques in overcoming segmental nerve defects in cats. Nerve biopsies from the 6 rhesus monkeys have been obtained and extensive light and electron microscopic evaluation continues. The cynomologus monkeys will have final evaluation 5 months after their neurorrhaphies.

*(Available to contractors upon originator's approval)

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 015 Animal Models for Surgical Repair of Musculoskeletal Structures

The following investigations have been conducted under this work unit:

STUDY NO. 1 Nerve repair in cats: grafts vs tension
Nerve repair in Cynomologus macaque monkeys

STUDY NO. 2 Digital extensor tendon repairs in monkeys

STUDY NO. 1. The ulnar nerve of the domestic cat was used as a model for repair of lacerated peripheral nerves. Sixteen cats underwent bilateral ulnar neurorrhaphy after a 2 cm segment of the nerve was removed. By using microsurgical methods, one side was repaired by an epineurial technique under tension, and the other side was repaired by using multiple sural nerve grafts. All cats were evaluated for return of function 6 months following nerve repairs. There was no statistical difference between these 2 techniques in overcoming segmental nerve defects in cats, which suggests that moderate tension is neither worse, nor better, than inserting avascular grafts. The results of either technique were not as good as those seen with end-to-end repair of a nerve when no segment existed. The rate and morphometric pattern of axon regrowth are being examined by sequential biopsies of the ulnar nerves of rhesus monkeys at weekly intervals following neurorrhaphy.

The ulnar nerve of the Cynomologus monkey was used as a model for repair of lacerated peripheral nerves. Sixteen monkeys underwent bilateral ulnar nerve transsection and resection of 0, 1, 2, or 3 cm of the ulnar nerve in the mid forearm. By using our microsurgical techniques, one side was repaired by a standard epineurial technique under tension and the contralateral side was repaired by using multiple interfascicular sural nerve grafts. Five months after the neurorrhaphies, the animals were evaluated for return of function. Evaluation included axon counts proximal and distal to the neurorrhaphies, as well as in the mid-graft segment on the grafted side and in the appropriately innervated digital nerves in the hand. Additional evaluation included the weights of the reinnervated intrinsic muscles of the hand and histologic evaluations of the neuromas and reinnervated muscles. Clinically, all neurorrhaphies healed and produced reinnervation of the hand intrinsic muscles and currently the axon counts and histologic studies are being accomplished. The preliminary results indicate that more important than the type of repair is the amount of nerve tissue lost during the initial injury in determining the end result following neurorrhaphy.

STUDY NO. 2. Twenty-four adolescent and three skeletally mature rhesus monkeys underwent common digital extensor tendon transection and repair in the mid-metacarpal region of the index and little fingers. Regardless of the period or method of immobilization, tenodesis occurred in none of the repaired tendons. Consequently, it has not been possible to determine the optimal period of immobilization when this animal model is used. Regardless of the period of immobilization, tensile strength did not start to increase appreciably until the 21st postoperative day. An excellent histologic study of the healing processes of repaired extensor tendons has now been completed.

BODY OF REPORT

WORK UNIT NO.	015	Animal Models for Surgical Repair of Musculoskeletal Structures
STUDY NO.	1	Nerve repair in cats: grafts vs tension Nerve repair in Cynomologus macaque monkeys

PROBLEM

Peripheral nerve injuries are common in both combat and noncombat military accidents. Many of the war injuries from the Vietnam conflict included severe damage to the peripheral nerves of the upper and lower extremities. During one 24-month period, 54% of all casualties in military hospitals had such injuries. Although our technical capabilities in the surgical repair of peripheral nerves have progressed greatly during the last several years, we still do not have a good method of managing segmental nerve defects. Tension at the repair site is considered detrimental to nerve regeneration and healing. Consequently, the use of a multiple nerve graft has been advocated. Problems of repairing a nerve under tension, where joints must be flexed, nerves must be mobilized, and vascularity is diminished, are not completely overcome by the use of multiple nerve grafting procedures in which an avascular unmatched segment is used to bridge the defect and relieve tension. Intrafascicular grafting not only results in the interposition of an avascular segment which loses all endoneurial elements and structure, but this technique also requires 2 separate neurorrhaphies which regenerating neurites must cross. We know of no evaluation comparing nerve repairs with tension to those neurorrhaphies which have been done with the use of multiple grafts. These studies critically compare, by objective evaluation, epineurial end-to-end repairs with tension to interfascicular grafts without tension following loss of a nerve segment.

RESULTS AND DISCUSSION OF RESULTS

We have previously described an experimental model for peripheral nerve repair by using both ulnar nerves of domestic cats. In the present study, 16 house cats underwent bilateral resection of a 2-cm length of the ulnar nerve proximal to the medial humeral epicondyle. One nerve was sutured under tension with size 8-0 nylon by using an epineurial technique. The other nerve was repaired by using a multiple caudal cutaneous sural graft that eliminated all tension at both suture lines. Size 10-0 nylon was used to suture the grafts. Microsurgical technique was used for all nerve repairs. Six months after the nerve sutures, cats were evaluated for comparison of return of function. Subjective

evaluation included observation of gait, ability to fan claws (intrinsic function), and withdrawal from pin prick (sensation). Objective evaluation included efficiency and maximum strength of the ulnar innervated flexor muscles, weight of the flexor carpi ulnaris muscle, and regrowth of myelinated nerve fibers by total axon counts proximal and distal to the repairs.

Evaluations have been completed and statistically analyzed. There was no statistical difference between these 2 techniques in overcoming segmental nerve defects in cats; these findings suggest that moderate tension is no worse, nor better, than inserting avascular grafts. When compared to an initial study where nerves were repaired primarily without tension, we found that all animals with segmental defects had less return of function than those animals which had no segmental defect but merely an acute laceration and end-to-end repair. To determine the rate and morphometric pattern of axon regrowth following nerve laceration and repair, the ulnar nerves of 6 rhesus monkeys were severed and repaired primarily. At one-week intervals beginning 7 days after the neurorrhaphies, the nerves were biopsied and prepared for light and electron microscopic examination. Analysis of these sections has demonstrated that axon sprouting begins immediately after transection and repair with the neurite sprouts passing rapidly into the distal stump. The sequential nature of the Wallerian degeneration has been well demonstrated, and the regrowth and remyelinization of the axon sprouts are clearly demonstrated by the series of electron photomicrographs.

Sixteen Cynomologus macaque monkeys underwent resections of 0, 1, 2, or 3 cm of both ulnar nerves in the mid forearm. On one side, a repair was accomplished (using 8-0 nylon) by standard epineurial technique under varying amounts of tension as determined by the amount of defect. The contralateral nerve was repaired by using multiple sural cutaneous nerve grafts that eliminated all tension at both suture lines. Size 10-0 nylon was used to resuture the grafts. Microsurgical technique using appropriate magnification was used for all nerve repairs. Five months after the nerve sutures, the monkeys were evaluated for comparison of return of function. Subjective evaluation included inspection of the neuromas and stimulation of the ulnar nerves proximal to the neurorrhaphies, and evaluating the amount of contraction in the hand intrinsic muscles. Objective evaluation included weights of the ulnar innervated hypothenar intrinsic muscles in the hands, as well as the axon counts of myelinated nerve fibers proximal and distal to the neurorrhaphies and in the reinnervated digital nerves in the ring and little fingers.

Objective evaluations have been completed and all neurorrhaphies had healed and had produced sufficient reinnervation to allow no detectable difference in gross contraction of the ulnar innervated intrinsics.

The histologic studies on the neuromas and the hand intrinsic muscles as well as the axon counts on the ulnar nerves and digital nerves are currently in progress.

CONCLUSIONS

Although we do not as yet have a satisfactory answer to the management of segmental defects of peripheral nerves, we have demonstrated that nerves repaired without tension, when compared to those with segmental defects, have a greater return of function. Based on the electron microscopic study of nerve regeneration, it appears that neurite sprouting occurs immediately and without a significant delay, as has been proposed historically. From these findings we conclude that the most ideal nerve repair is one performed as soon as possible after the injury, without tension, without grafts, with atraumatic technique, and with appropriate alignment of the fascicular nerve ends.

Although the objective data evaluation is not complete in the Cynomologus study, it appears that the most important factor in determining the end result is not the surgical technique, but rather the amount of segmental defect produced at the time of injury. From this study it appears that, depending on the amount of segmental defect, a surgical technique should be utilized that allows the surgeon to accomplish the neurorrhaphy as rapidly and securely as possible and to meet the previously established criteria of performing a neurorrhaphy as soon as possible after the injury without tension, with grafts only if necessary, with atraumatic technique, and with appropriate alignment of the fascicular nerve ends.

RECOMMENDATIONS

The extensive amount of data prepared on the electron microscopic study on regenerating nerves should be assimilated and prepared for presentation and publication. Since all the data and specimens have been collected from the Cynomologus study, it is critical to complete the objective evaluations and prepare the information for presentation and publication. Further problems on the management of peripheral nerve injuries will undoubtedly be generated from the results of the current study.

PUBLICATIONS

1. RODKEY, W.G., H.E. CABAUD, and H.R. McCARROLL. Neurorrhaphy after loss of a nerve segment: Comparison of epineurial suture under tension versus multiple nerve grafts. J Hand Surg, in press.

2. CABAUD, H.E., W.G. RODKEY, and H.R. McCARROLL. Peripheral nerve repairs. Studies in higher non-human primates. J Hand Surg, in press.

3. RODKEY, W.G., H.E. CABAUD, and H.R. McCARROLL. Neurorrhaphy after loss of a nerve segment: Comparison of epineurial suture under tension versus multiple nerve grafts (abstract). J Hand Surg 4: 283, 1979.

4. RODKEY, W.G., H.E. CABAUD, and H.R. McCARROLL. Neurorrhaphy after loss of a nerve segment: Comparison of epineurial suture under tension versus multiple nerve grafts (abstract). Orthop Trans 3: 324, 1979.

5. McCARROLL, H.R., W.G. RODKEY, and H.E. CABAUD. Results of suture of cat ulnar nerves: A comparison of surgical techniques. In: Nerve Repair and Regeneration: Its Clinical and Experimental Basis, edited by D.L. Jewett and H.R. McCarroll, Jr. St. Louis: Mosby, 1980. pp 228-234

STUDY NO. 2 Digital extensor tendon repairs in monkeys

PROBLEM

Study of tendon injuries and their repair has centered around flexor tendons. Lack of attention to extensor tendon injuries may be attributed to the fact that extensor tendons are the positioners of the hand and fingers, and these tendons serve as stabilizers so that the flexors can perform the primary functions of pinch, grasp, and hook. Consequently, there is a paucity of published data regarding healing of extensor tendons, even though extensor tendon injuries are common in both combat and noncombat situations. When dealing with a clean, sharp transection of an extensor tendon, it is general practice to reapproximate the severed ends surgically and immobilize the injured part during healing. The optimal duration of immobilization, however, is subject to great controversy. Prolonged immobilization of a repaired extensor tendon may result in increased scar formation, tenodesis, and contracture. This may result in a temporary or permanent disability of the part served by the tendon, and additional surgical procedures such as tenolysis may be required to restore mobility to the tendon. Conversely, use of a repaired extensor tendon too soon after repair may result in failure. Again, this leads to additional surgical procedures to correct the problems. This study has been designed to determine the optimal period of immobilization of a repaired extensor tendon, and to help identify and describe the cellular processes involved in healing of extensor tendons repaired after transection.

RESULTS AND DISCUSSION OF RESULTS

As previously discussed, we have described the histologic appearance of the healing extensor tendon. We have also found that the healing extensor tendon of the rhesus monkey does not develop appreciable strength until after 21 days following surgery. However, we have not been able to determine accurately the optimal period of immobilization following extensor tendon repair because all experimental groups healed in the same satisfactory manner, and no tenodesis developed in any of the repaired tendons regardless of the length and method of immobilization. Consequently, we attempted to produce a tenodesis by using animals in an older age group, but our attempts were still unsuccessful.

CONCLUSIONS

We feel that our model (Cynomologus macaque monkeys) is not adequate to evaluate the optimal period of immobilization of a repaired extensor tendon. Consequently, further attempts to make such determinations will not be continued in this model.

RECOMMENDATIONS

The final report of the histologic appearance of the healing extensor tendons has been completed, but has not been accepted for publication. No further studies will be conducted using this animal model (Cynomologus macaque monkeys).

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6309	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 15	D. CHANGE		U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	016 APC 505H
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Studies in Combat Fracture Healing

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 79	2.0	43
FISCAL YEAR CURRENT 80	3.0	63

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Combat Injuries; (U) Fractures; (U) Ligamentous Injuries; (U) Trauma; (U) Surgery

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Fractures and ligamentous injuries due to combat frequently result in delayed healing and permanent disability. Prolonged hospitalization and multiple surgical procedures delayed return to duty, and eventual medical separations are common sequelae to such injuries. Multiple systemic and mechanical factors are known to retard fracture and ligament healing, but considerable controversy still exists about how fracture and ligament healing can be accelerated. Under this work unit biochemical alterations and various surgical modalities will be investigated. The results will be transferred into management principles and techniques for combat fracture healing.

24. (U) Twelve dogs underwent acute anterior cruciate ligament repair with augmentation utilizing the medial third of the patellar tendon. They were evaluated for function and mechanical strength at 4 and 8 months after the repairs. Rats were exercised at endurance levels of differing frequency and duration for the exercise regimens and then the anterior cruciate ligaments were tested for changes in tensile strength.

25. (U) 78 10 - 79 09 All repaired and augmented anterior cruciate ligaments healed and provided normal function and satisfactory strength. The ligaments tested at 8 months were stronger than those tested at 4 months. All exercise regimens proved beneficial to the strength and stiffness of the rat anterior cruciate ligament, but those exercised at high frequency (daily) and lower duration (30 minutes rather than 60 minutes) had the greatest increase in strength and stiffness.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 016 Studies in Combat Fracture Healing

The following investigations have been conducted under this work unit:

STUDY NO. 1 Effects of electrical stimulation on experimental ununited fractures and the establishment of nutritional supplemental controls in accelerating fracture healing

STUDY NO. 2 Evaluation of potential combined effects of electrical stimulation and nutritional attitudes in accelerating fracture healing

STUDY NO. 3 Evaluation of repair techniques in treating avulsion fractures and injuries of the anterior cruciate ligament

EX-4 The effect of exercise and interval training upon anterior cruciate ligament strength

STUDY NO. 1. Electrical stimulation stimulates hydroxyapetite formation and facilitates healing of experimentally produced fractures. Clinically, electrical stimulation has been used to accelerate healing of delayed or ununited fractures. An experimental nonunion model utilizing the canine tibia allows controlled investigation of the role of electrical stimulation on ununited fractures. Similarly, active metabolites of vitamin D, calcitonin, and fluorides are known to affect bone formation, and therefore have a potential effect on fracture healing. The effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, calcitonin, fluorides, and excess phosphate have been evaluated for their effects in accelerating or altering fracture healing in feline fracture models.

STUDY NO. 2. Since electrical stimulation stimulates the healing of experimental ununited fractures and osteotrophic agents accelerates fracture healing, the combined effects of electrical stimulation and such agents are being evaluated. The selection of appropriate agents will be based on the results of Study No. 1 of this protocol.

STUDY NO. 3. Eleven dogs underwent transection of the anterior cruciate ligament at the femoral origin of one stifle joint. The anterior cruciate ligaments were repaired in a conventional manner and augmented by transferring the medial one-third of the patellar tendon and inserting it into the lateral femoral condyle. The repairs were evaluated 4 and 8 months postoperatively. All repaired and augmented anterior

cruciate ligaments in this series healed satisfactorily to provide clinical and functional stability of the knee joints. Histologic evaluation showed that by 8 months the repaired and augmented anterior cruciate ligaments had healed by bony ingrowth, thus interstitial failure occurred during failure testing. The transferred patellar tendon provided additional blood supply, splinted the anterior cruciate ligament to allow healing, and increased the strength of the repaired complex.

STUDY NO. 3, EX-4. Seventy-five rats were divided into a control and 4 exercise groups of differing frequency and duration. After 8 weeks of endurance-type exercise on a motorized treadmill, the rats were sacrificed and the anterior cruciate ligaments were tested to failure. This study has shown that endurance-type exercise is beneficial to the anterior cruciate ligament as both strength and stiffness are increased, and functionally the ligament remains unchanged by the exercise.

BODY OF REPORT

WORK UNIT NO.	016	Studies in Combat Fracture Healing
STUDY NO.	1	Effects of electrical stimulation on experimental ununited fractures and the establishment of nutritional supplemental controls in accelerating fracture healing

PROBLEM

A significant percentage of combat and noncombat injuries are fractures. These injuries not only require front line treatment but often require rear area surgical procedures and prolonged hospitalizations. Due to the adverse environments of combat zones and the unique nature of such combat-incurred fractures, delayed healing and permanent disability often result. Since electrical stimulation has been shown to stimulate fracture healing and the healing of delayed or ununited fractures, it is the purpose of this investigation to evaluate the role of electrical stimulation in accelerating fracture healing and preventing ununited fractures. Various osteotrophic agents have been implicated in stimulating fracture healing, and deficiencies of these agents have resulted in delayed fracture healing. This study also will evaluate the potential roles of such agents, including vitamin D, calcitonin, fluorides, and excess phosphate (reversed calcium-phosphate ratio) in accelerating or altering fracture healing and preventing ununited fractures.

RESULTS AND DISCUSSION OF RESULTS

Prototypical studies with canine tibiae have resulted in the development of a bilateral experimental nonunion model. Other techniques for developing nonunion models have been described, have been used in similar studies, and have shown that electrical stimulation does accelerate fracture healing in nonunions.

Thirty-six domestic house cats underwent bilateral midshaft ulnar osteotomies and were divided into 6 test groups for evaluation of osteotrophic agents. The study groups included a control group, a pathologic group with excess phosphate, and the following experimental groups: $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, fluoride, and calcitonin. Twelve weeks after the osteotomies, the animals were evaluated for fracture healing, bone mineral content, serum chemistry, and alterations in calcium phosphorus metabolism.

CONCLUSIONS

Evaluation of data obtained in the cat fracture study has indicated that none of the osteotrophic agents accelerated or altered the rate

of fracture healing in any of the experimental groups. Although slight statistical differences were noted in the serum chemistries and bone mineral content between the various groups, none of these changes could be correlated with clinical relevance.

RECOMMENDATIONS

Although no significant changes were noted in the fracture healing in this particular experimental model, perhaps the development of another type of fracture model would be beneficial in evaluating the effects of various osteotropic agents.

PUBLICATIONS

None

STUDY NO. 2 — Evaluation of potential combined effects of electrical stimulation and nutritional attitudes in accelerating fracture healing

PROBLEM

Since it has been shown that electrical stimulation accelerates fracture healing with mechanical strength increased approximately 30%, it is possible that substrates and osteotrophic agents may become the rate-limiting factor if they are not available in adequate amounts. In Study No. 1, the potential roles of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, calcitonin, fluorides, and excess phosphate in accelerating or altering fracture healing have been evaluated. If it can be shown statistically that one of these agents accelerates fracture healing, then the combination of that agent with electrical stimulation may further accelerate fracture healing so as to provide earlier return to duty for the combat injured soldier and to reduce the incidence of delayed or ununited fractures.

RESULTS AND DISCUSSION OF RESULTS

Since the results of Study No. 1 are inconclusive, no work has been done on this study.

CONCLUSIONS

None

RECOMMENDATIONS

The potential benefits of electrical stimulation in fracture healing has been well demonstrated in other publications and by the commercial availability of electrical stimulation for healing fractures, and therefore the potential benefit of electrical stimulation should be considered in further work units.

PUBLICATIONS

None

STUDY NO. 3 — Evaluation of repair techniques in treating avulsion fractures and injuries of the anterior cruciate ligament

PROBLEM

Incompetence of the anterior cruciate ligament and the resulting rotatory instability of the knee is a militarily devastating handicap. A significant percentage of soldiers who sustain anterior cruciate ligament injures in training or combat develop knee instability require medical separation regardless of methods of treatment. Although excellent functional, anatomical, and biomechanical studies of the anterior cruciate ligament have been reported, there is still considerable disagreement as to whether a ruptured or avulsed anterior cruciate ligament should be repaired, discarded, replaced, or ignored. Based on our results in our initial study, where primary repairs were accomplished in the proximal and distal portion of the anterior cruciate ligament, this current study will evaluate the results of primary repairs of the anterior cruciate ligament augmented with the medial third of the patellar tendon. Based on the results of supplemental autogenous grafting in the current studies, we will evaluate the role of synthetic materials in repairing or replacing the injured anterior cruciate ligament.

RESULTS AND DISCUSSION OF RESULTS

Eleven dogs underwent transection and repair of the anterior cruciate ligament at the femoral origin. The ligament was augmented by transferring the medial one-third of the patellar tendon and inserting it into the lateral femoral condyle. The repairs were evaluated at 4 and 8 months postoperatively, and all repaired anterior cruciate ligaments healed clinically providing excellent functional stability in all animals. Instron testing of the repaired and augmented anterior cruciate

ligaments showed maximum strength at 4 months of 46.2 ± 10.9 kg_f and at 8 months of 64.3 ± 14.3 kg_f as compared to the control of 122.7 ± 11.6 kg_f. Histologic evaluation showed that by 8 months the repaired and augmented anterior cruciate ligaments had healed by bony ingrowth, thus interstitial failure occurred during Instron testing.

CONCLUSIONS

It is apparent that the transferred patellar tendon has provided the repaired anterior cruciate ligament with the opportunity to heal and regain functional competence. Additional blood supply was grossly evident with new blood vessels passing from the patellar tendon to the anterior cruciate ligament. The transferred patellar tendon has acted as an internal splint to provide support and knee joint stability while the cruciate has healed. We believe that early stress on a repaired anterior cruciate ligament may be extremely deleterious. Finally, in this study the transferred patellar tendon has truly augmented the strength of the anterior cruciate ligament with one repaired complex being stronger than the opposite control anterior cruciate ligament.

RECOMMENDATIONS

Based on the encouraging results of these augmented primary repairs, in the dogs in this study, we believe that augmenting acutely injured and repaired anterior cruciate ligaments with the medial third of the patellar tendon is appropriate for clinical trials at this time. Using the model and surgical technique that has been developed, we recommend that a synthetic cruciate ligament be used to splint and augment the anterior cruciate ligament. These studies utilizing the synthetic cruciate ligament have been started; further evaluation may show improved long-term results from repaired anterior cruciate ligaments.

PUBLICATIONS

1. CABAUD, H.E., W.G. RODKEY, and J.A. FEAGIN. Experimental studies of acute anterior cruciate ligament injury and repair. Am J Sports Med 7: 18-22, 1979.

2. CABAUD, H.E., W.G. RODKEY, and J.A. FEAGIN. Experimental studies of acute anterior cruciate ligament injury and repair (abstract). Orthop Trans 3: 98, 1979.

3. CABAUD, H.E., J.A. FEAGIN, and W.G. RODKEY. Acute anterior cruciate ligament injury and augmented repair. Experimental studies. Am J Sports Med, in press.

4. CABAUD, H.E., W.G. RODKEY, and J.E. FITZWATER. Medial meniscus repairs: An experimental and morphological study. Submitted for publication.

STUDY 3, EX-4 — The effect of exercise and interval training upon anterior cruciate ligament strength

PROBLEM

Incompetence of the anterior cruciate ligament and the resulting rotatory instability of the knee is a militarily devastating handicap. Exercise has been shown to increase the strength of certain static ligaments supporting the knee joint, particularly the collateral ligament. Since the anterior cruciate ligament is particularly susceptible to injury and often requires surgical repair, it is imperative to determine whether or not exercise and interval training would strengthen the anterior cruciate ligament and therefore protect it against injury. A significant percentage of soldiers who sustain anterior cruciate ligament injuries in training or combat develop knee instability require medical separation regardless of methods of treatment.

RESULTS AND DISCUSSION OF RESULTS

Seventy-five rats were divided into a control and four exercise groups of differing frequency and duration. After 8 weeks of endurance-type exercise on a motorized treadmill, the rats were sacrificed and the anterior cruciate ligaments were tested to failure on an Instron Materials Testing Machine at a strain rate of 95% per second. Of the 121 ligaments tested, 119 failed by pure interstitial failure. There was significant increase in both the strength and stiffness of the anterior cruciate ligaments in the exercised rats, but those rats exercised more frequently (daily versus every other day) and for shorter duration (30 minutes rather than 60 minutes) had the greatest increase in strength.

CONCLUSIONS

We can conclude from this study that 1) endurance-type exercise has a generally positive effect on the strength and stiffness of the anterior cruciate ligament; 2) the greatest increase in strength and stiffness is produced from high frequency, low duration exercise and the minimum changes from low frequency, high duration exercise; and 3) independently longer or less frequent exercise may decrease the generally positive increase in strength and stiffness that develops after daily short-duration endurance exercise.

RECOMMENDATIONS

Based on the findings in this study, we suggest that perhaps it would be appropriate to change training regimens for both athletes and soldiers in efforts to provide the most significant increases in strength for their static supporting ligaments of the knee joint. Certainly, rehabilitation programs following surgical repair of the anterior cruciate ligaments should be modified to reflect the positive increases in strength that are found with high frequency, low duration exercise. Additional studies are needed to evaluate further the effects of exercise on the strength of ligaments.

PUBLICATIONS

1. CABAUD, H.E., A. CHATTY, V. GILDENGORIN, and R. FELTMAN. The effect of exercise on the strength of the rat anterior cruciate ligament. Am J Sports Med, in press.

2. CABAUD, H.E., A. CHATTY, and V. GILDENGORIN. Anterior cruciate ligament of the rat. Effects of exercise on strength. (Abstract) Orthop Trans 3: 360, 1979.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6317	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GRP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 08 15	D. CHANGE		U	NA	NL	X ☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62172A	3S162172A814	00	018
b. CONTRIBUTING				
c. CARDS	CARDS 114f			

11. TITLE (Provide with Security Classification Code)

(U) Development of Optimal Blood Products

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 10	83 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 79	6.5	163
FISCAL YEAR CURRENT 80	7.0	166

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME: Bolin, Robert B., LTC, MC POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) CPD Adenine; (U) Blood Storage; (U) ATP; (U) Glucose; (U) 2,3-DPG

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Provide text of each with Security Classification Code.)

23. (U) Forward resuscitation of the wounded soldier requires that front line medical units maintain an adequate supply of viable, functional whole blood or packed red cells. This inventory must be available in spite of large fluctuations in usage, delays, limitations, or interruptions in normal supply lines. This dictates that stored blood products have the longest possible shelf life and be of the highest quality. This work unit addresses the development of extended liquid storage of blood products (42-100 days) as well as the improvement of the oxygen transport function of the stored blood.

24. (U) Preservative chemicals known to improve red cell adenosine triphosphate (ATP) and 2,3 diphosphoglycerate (2,3-DPG) will be evaluated singly and in combination using modern optimization techniques. Maximally effective formulations of citrate phosphate dextrose (CPD) adenine and optimal additive systems will be developed. The 2,3-DPG maintenance problem will be studied and the membrane integrity limits of long term liquid storage defined.

25. (U) 78 10 - 79 09. A strategy has been developed for the specific preservation of red blood cells using the addition of specific metabolic cocktails after component preparation. This is termed the optional additive system (OAS) approach. All OAS solutions tested contained glucose and adenine to maintain cell viability during 42 days of storage, as well as dihydroxyacetone, ascorbate phosphate, or combinations thereof to maintain 2,3-DPG for optimal oxygen off-loading characteristics.

*Available to contractors upon originator's approval.

DD FORM 1498 (1 MAR 68) PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 018 Development of Optimal Blood Products

In vitro evaluation of CPDA-2 (1.75 x glucose of CPD + 0.5 mM adenine) as an anticoagulant solution for red cell storage up to 56 days has been completed. The results indicated that that shelf-life of red cells stored in CPDA-2 could be extended beyond 35 days. Optional additive systems (OAS) are being developed. Specific solutions containing adenine, glucose, dihydroxyacetone, and/or ascorbate phosphate have been tested. In vitro, these studies suggest a system that is superior to any of the CPD-adenine formulations because it provides not only for extended storage but also for improved oxygen delivery. The OAS also allows for the preparation of blood components free of unneeded chemical additives. Studies of the OAS solutions demonstrate the capability to store packed red cells for at least 42 days with a viscosity that does not require dilutions before infusion. The OAS also significantly improves functional quality over other systems. Functional quality is defined as maintenance of red cell 2,3-DPG (and associated P_{50}) with improved oxygen off-loading properties in vitro.

BODY OF REPORT

WORK UNIT NO. 018 Development of Optimal Blood Products

PROBLEM

Military blood banking differs from its civilian counterpart because of unique logistical limitations imposed in combat situations. In a civilian setting blood is drawn, stored under "ideal" conditions, and used in a geographically contained community at a relatively predictable rate. Under these conditions, blood shortages are minimal and loss due to outdating is less than 10%. The wartime use of blood in the Army may be illustrated by the Vietnam experience, which is probably a best-case example. The blood used in Vietnam was drawn in CONUS and had CPD anticoagulant added. It had a 21-day dating period. The time required to process and ship this blood to field medical units was 7 to 14 days, which left only 7 to 14 days of shelf life remaining. Due to limited shelf life and the large fluctuation in casualty rate, outdating was possibly as high as 50%, while inventories were dangerously low in many instances. These problems could have been largely overcome if the shelf life of blood had been 35 or 42 days. In future conflicts, the U.S. may not have air superiority, thus logistical problems in all areas of supply, including fresh blood and blood products, will be compounded. To support the wounded soldier with available blood products, it will be imperative to be able to store blood for extended periods of time. In addition, it is essential that the stored blood maintains its functional qualities. These ends can be met by the development of new systems for blood storage that extend the shelf life (viability) and improve the oxygen-delivering quality of red cells. A significant step in this direction was taken with the development of CPDA-1 anticoagulant which allows for the 35-day storage of whole blood or packed cells of hematocrit not over 80. New efforts are underway to extend blood storage beyond 35 days, and also to improve the quality of long-term stored blood. At this time, two specific studies are underway: a) optimization of CPD-adenine, and b) development of an Optional Additive System (OAS). The development of CPDA-1, while offering a significant improvement in blood storage, does not produce the optimum results in red cell storage that is attainable with a glucose-adenine mixture. Two new formulations of CPD-adenine (CPDA-2 and CPDA-3), which are closer to optimal for packed cell storage, are being evaluated in vitro.

The best approach to extended quality storage of red blood cells is by use of a specific solution for addition to packed red cells. This approach is termed an "Optional Additive System." Solutions are being developed and tested (in vitro) which allow for extended storage of packed red cells, and at the same time improve the functional quality of these cells by maintaining the concentration of red cell 2,3-DPG. The development of these systems will provide military blood banking with the

capability to a) store red blood cells to extended periods of time beyond 35 days, b) improve the functional qualities of these cells, i.e. their oxygen off-loading characteristics by maintaining normal P_{50}, and c) make available for separate use, fresh plasma components in maximum quantities, free of adenine or other additives.

RESULTS AND DISCUSSION OF RESULTS

Long-term storage (to 56 days) of red cells drawn in CPDA-2 or CPDA-3 anticoagulant solution was evaluated in vitro for whole blood and hard packed cells (Hct = 85). CPDA-2 contains 1.75x the glucose of CPD and 2x the adenine of CPDA-1; the CPDA-3 solution contains 2x the glucose of CPD and 2x the adenine of CPDA-1. CPD units were also used as control; N = 5 for each anticoagulant and each hematocrit. Packed cell units were held 8 hours before processing, as advised by Dr. Ernest Simon, Chief of Blood Products Section, Bureau of Biologics, Food and Drug Adminstration. Measurements of red cell ATP, which correlate with cell survival, can be used as an in vitro indicator of cell quality. A stored red cell mean ATP level of at least 45% of the initial value is the criterion for expecting 70% mean survival upon infusion. In this study, both CPDA-2 and CPDA-3 whole blood units retained greater than 60% of initial ATP out to 56 days of storage. Packed cell units, drawn in either CPDA-2 or CPDA-3, had mean ATP levels of 45% by day 56 of storage. Glucose loads in both anticoagulants were adequate, since the day 56 blood glucose concentrations were between 100-200 mg/dl. Measurements of pH, red cell 2,3-DPG, and plasma hemoglobin were similar to those seen in CPDA-1. The data indicate that CPDA-2 and CPDA-3 produce identical results, but both are superior to CPDA-1 for storage of red cells to 56 days. The time limit of packed cells appears to be 49 to 56 days in CPD-adenine systems. The development of optional additive systems for blood storage has evolved around the concept of drawing blood in basic CPD, processing it into components, then adding to the red cell an OAS solution to provide for (1) extended storage, (2) improved function by 2,3-DPG maintenance, (3) and improved flow properties by dilution of red cells to Hct < 70%. Extended shelf life has been addressed by including adenine and extra glucose in the OAS solution; the solution itself serves to reduce hematocrit and lower viscosity.

The problem of maintaining 2,3-DPG to assure proper oxygen off-loading to tissues has been evaluated with addition of dihydroxyacetone (DHA) and/or ascorbate-2-phosphate (AsP) to the OAS solutions. These work in an opposite sense on ATP and 2,3-DPG maintenance. Therefore, the optimum concentration of each component must be evaluated to attain the best balance between ATP and 2,3 DPG. This is being done by factorial experiments. The preliminary results indicate that levels of glucose and adenine, equivalent to those seen by red cells in CPDA-2 whole blood will maintain red cell ATP above 45% for 42 days in the presence of DHA

or AsP. When 30 mM DHA is used in OAS solutions the 2,3-DPG levels are 70% of normal at 21 days of storage, with an almost linear fall to 20% by 42 days. In CPD controls the 2,3-DPG concentrations are below 20% by day 21. The use of 5 mM AsP causes a rise of 2,3-DPG to 160% normal by 21 days and then a fall to 80% normal by day 42. However, AsP causes a negative effect on ATP by reducing it to 50% normal in 3 days, and 30% by 42 days. Thus, a critical evaluation of the ATP-survival relationship in this system is required. A fine-tuned mixture of adenine, glucose, DHA, and AsP has been developed that can be added on day zero or day 7. This solution yields mean ATP values of 45% on day 42 and 2,3-DPG concentrations of 120% on day 14, which fall to 45% by day 42. The success of DHA or AsP in a commercial blood bag package may depend on their stability in the bag between the time of manufacture and use which is normally controlled by a 3-year dating period. The stability of DHA solutions in water or saline with/without other OAS additives is being evaluated. Sterile fill and autoclaving techniques are being compared. Preliminary results indicate that DHA is stable in saline or with non-ionizing non-phosphate OAS compounds. After 7 months storage, the autoclave samples show slight deterioration (< 10%) when compared to sterile-fill samples. These studies are continuing. Similar studies for 3 months with AsP indicated that the AsP was stable in OAS solutions. These studies were terminated due to personnel losses.

One disadvantage of long-term red cell storage is the increase (to several hundred mg/dl) of plasma hemoglobin. It is speculated that this problem is related to white cell protease activity on the red cells. The addition of E-amino caproic acid (EACA), a protease inhibitor, to the OAS solution has resulted in up to a 80% reduction in hemolysis. EACA is not proposed as an additive since its use in massive transfusions might prove toxic, but it serves as a model against which to test other procedures such as in-line cellulose filtration to remove white cells.

CONCLUSIONS

CPDA-2 is a superior CPD-adenine formulation when compared to CPDA-1 and appears to be close to "optimal" for long-term red cell storage in which adenine and extra glucose are used in the anticoagulant.

Optional additive solutions have been developed and tested (in vitro) which indicated a capability of providing viable red cells for 42 days of storage, and also of significantly improving the oxygen off-loading characteristics of these cells. This functional improvement is extremely important during the first few hours postinfusion in the massively transfused soldier. Certain refinements need to be made in the system, such as reduction of plasma hemoglobin levels. Discussion of OAS with the civilian blood community has generated great interest.

Development of Optimal Blood Products

RECOMMENDATIONS

CPDA-2 should be evaluated by clinical trials, and if the results are superior to CPDA-1, CPDA-2 should replace CPDA-1 as the standard adenine containing anticoagulant. Optional additive systems for blood storage offer advantages that make them clearly superior to CPD-adenine anticoagulant formulations and should be further evaluated (in vitro) before designing clinical trials.

PUBLICATIONS

1. MOORE, G.L., M.E. LEDFORD, and D.E. BROOKS. The distribution and utilization of adenine in red cells during 42 days of 4 C storage. Transfusion 18:538-545, 1978.

2. MOORE, G.L., M.E. LEDFORD,and D.E. BROOKS. Plasma adenine and cellular ATP in red cell concentrates collected and stored in modified CPD at 4 C. Transfusion 19:68-71, 1979.

3. MOORE, G.L. Different mean red cell organic phosphate concentrations in black versus white donors. Letters to the Editor. Transfusion 18:767-768, 1978.

4. MOORE, G.L., M.E. LEDFORD, M.R. BRUMMELL, and D.F. BROOKS. The potential use of dihydroxyacetone for improved 2,3-DPG maintenance in red cell storage. Transfusion 20:24, 1980.

5. LEDFORD, M.E., and G.L. MOORE. The formulation of citrate-phosphate-dextrose anticoagulant. Letters to the Editor. Transfusion 19:350, 1979.

6. MOORE, G.L., M.E. LEDFORD, and C.C. PECK. The in vitro evaluation of modifications in CPD-adenine anticoagulated-reserved blood at various hematocrits. Transfusion, 1980, in press.

7. PECK, C.C., G.L. MOORE, and R.B. BOLIN. Adenine in blood preservation. CRC Reviews in Clinical Laboratory Science, 1980, in press.

8. PECK, C.C., G.L. MOORE, V. LICKO, R.B. BARRETT, and L. LENERT. The design of optimal blood preservation systems. Clin Res, 1980, in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6090	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. SUM'R HIST'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	019 APC 505N
b. CONTRIBUTING				
XXXXXXXXXX	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Investigation of Cell-Free Resuscitating Solutions

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support; 003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 03	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING: 79	2.9	165
FISCAL YEAR CURRENT: 80	6.0	203

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Scannon, Patrick J., MAJ, MC

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Acute Resuscitation; (U) Stroma-Free Hemoglobin; (U) Blood Substitute Solutions; (U) Hemorrhagic Shock

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of these studies is to evaluate the effectiveness of hemoglobin solution as a resuscitating fluid for military use. Hemoglobin free of cell constituents can provide the basis for an ideal resuscitating fluid for the severely wounded soldier. It has several advantages as compared to other blood substitutes or plasma expanders. It is capable of in vivo on-loading and off-loading oxygen with sufficient efficiency to maintain oxygen consumption in experimental animals rendered virtually free of circulating red cells. Hemoglobin can be stored for an extended time, thus alleviating logistic problems in fluid therapy of mass casualties in combat situations.

24. (U) Hemoglobin, prepared by crystallization from outdated human red cells, is being evaluated as a cell-free resuscitating solution in animal models for its ability in maintaining vital functions. Formulations of solutions and modifications of the hemoglobin molecule are being investigated.

25. (U) 78 10 - 79 09 Lyophilized hemoglobin requires purified sterile water for reconstruction to a solution. Field production of purified sterile injectable water has been investigated. A 60Kg reverse osmosis apparatus is capable of producing the required injectable water from available water sources. Multiple infusions of hemoglobin solution, by restoring plasma hemoglobin and intravascular fluid volume levels, prevent the hypoxic alteration observed in the liver of rats a few hours after a single massive transfusion with hemoglobin. Modifications of hemoglobin by polymerization or encapulation have not yet yielded a product with longer intrasvascular life and lower oxygen affinity.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 019 Investigations of Cell-Free Resuscitating Solutions

Recent research efforts have resulted in substantial improvements in the general purity and quality of experimental hemoglobin solution. The present product is suitable for prolonged storage and can be dehydrated for greater efficiency in shipping and stockpiling during emergencies. Lyophilized hemoglobin can be maintained for several months at room temperature and requires addition of sterile water for reconstitution to a solution. A compact reverse osmosis apparatus has been evaluated for the production of purified sterile injectable water in field situations. After massive transfusions, hemoglobin disappears rapidly from the circulation with an associated reduction in blood volume. These effects can be overcome by multiple infusions of hemoglobin solution. Plasma hemoglobin, blood volume, plasma oncotic pressure, and oxygen-carrying capacity are restored with each infusion. The hypoxic alterations observed in the liver several hours after a single massive transfusion with hemoglobin solution are thus prevented.

Several factors individually increase the yield of modified hemoglobin. These include hemoglobin and sugar concentrations, pH, type of sugar, and temperature. Hemoglobin has been reacted with a number of phosphorylated sugars in attempts to improve two major drawbacks of the current hemoglobin preparation, i.e., increased oxygen affinity and rapid plasma clearance. The phosphorylated sugars reduce methemoglobin formation. Oxygen affinity data from mixtures containing modified and unmodified hemoglobin show improved P_{50} values compared to unmodified hemoglobin alone. The selective chemical modification of hemoglobin with phosphorylated sugars has a promising role in improving unmodified hemoglobin for use in resuscitation solutions.

BODY OF REPORT

WORK UNIT NO.	019	Investigation of Cell-Free Resuscitating Solutions
STUDY NOS.	1, 2, 4, 5	Preparation of hemoglobin, in vivo evaluation, pharmacokinetics, and effects of hemoglobin on organs

PROBLEM

It has been known for a long time that, excluding damaging brain wounds, the majority of soldiers who are killed in combat die because of exsanguination. The basic medical problem is the need to restore blood volume; prompt treatment is important. Expeditious medical care of combat casualties was possible in Korea and in Vietnam, because hospitals were in close proximity to a stable front line and air superiority made helicopter evacuation possible. However, in a future war, air superiority may not be assumed and battlefield conditions may restrict evacuation. For this reason, the development of an effective resuscitating solution that can be administered in the battlefield is important.

As a resuscitating fluid, blood has limited storage life, must be stored in bulky energy-requiring refrigerators, and requires typing and crossmatching before use. Plasma, dextran, albumin and other preparations have been used as resuscitating fluids and, although they appear to be effective as plasma expanders, they do not carry oxygen. Significant advantages can be gained by the development of a resuscitating solution capable of transporting oxygen, maintaining oncotic pressure, and being readily available when massive transfusions are required. Stringent requirements must be met by a resuscitating solution in order to be effective. As a blood substitute, this solution not only must be capable of restoring vital functions, but also must not elicit permanent adverse effects when administered to patients. Furthermore, it must be suited to fulfill the supply, storage, and transportation requirements for field use in combat situations.

In most civilian settings in this country, the transfusion requirements associated with massive trauma can be met with conventionally stored blood and its components. However, military field requirements frequently demand massive fluid support in areas remote from supply sources. The inability to predict when modest transfusion requirements may suddenly increase complicates fluid therapy logistics. The ability to stockpile a stable protein solution capable of carrying and exchanging oxygen would minimize many of these difficulties.

Hemoglobin is a protein which has such potential. A solution of hemoglobin presents numerous advantages as compared to other blood substitutes

or plasma expanders. Hemoglobin is a component of normal blood, can be prepared from outdated human erythrocytes, does not require typing or cross-matching before use, is capable of transporting oxygen to the tissues, has oncotic activity, has low viscosity, does not cause micro-aggregates, may not induce significant immunologic reaction. Furthermore, hemoglobin is highly soluble in physiological solutions and can be stored for extended periods of time. The potential value of hemoglobin solution as an oxygen-carrying blood substitute has been recognized also in some special clinical situations.

It is imperative that if hemoglobin is used as a blood substitute, it must be free of any stromal particle, stromal lipid, or other soluble and insoluble cell components which have been implicated in adverse effects on kidney function and on coagulation factors. Also, two important limitations of the present preparation, namely, short vascular retention time and increased oxygen affinity must be overcome by processes involving modification of the hemoglobin molecule.

Hemoglobin has the potential to become an important blood substitute and could provide the basis for an ideal resuscitating solution for the severely wounded soldier. The problem of developing an effective blood substitute is pertinent not only to military combat casualties, but also to civilian casualties such as in accidents and mass disasters.

RESULTS AND DISCUSSION OF RESULTS

Water purification. In the development of hemoglobin solution as a blood substitute, the process of lyopholization was utilized (as discussed in previous annual reports) to reduce the hemoglobin solution to a dry powder for compact storage at room temperature. At the time of transfusion, the addition of sterile water would be necessary to reconstitute the lyophilized hemoglobin to a solution for fluid therapy.

In a normal urban environment, sterile water for injection is readily available; however, in field situations or in areas remote from supply sources, the procurement of purified water could present logistic difficulties. A 60 kg, compact, self-contained, portable water purification apparatus, adapted with a sterile micropore filter, has been evaluated for purification of sea water, pond water, and human urine, separately. The process is based on the reverse osmosis procedure and can use various power sources. The results indicate that polluted water can be purified by a single passage through the system, as demonstrated by considerable reduction of ions (less than 1 percent), and complete elimination of metal content and organic matter present in the sources of water. The water obtained is clear, colorless, odorless, nonpyrogenic, and sterile without addition of antimicrobial agents or other substances. It appears to satisfy the criteria for USP grade

water for injection except for the limits on total solids. The residual solids are sodium and chloride ions which are common constituents of parenteral solutions. This purification process is capable of meeting the medical needs of the military in its various fields of operation.

Multiple infusions of hemoglobin solution. Livers of rats transfused to a 75% blood replacement with plasmanate, albumin, or lactate Ringer's solution displayed evidence of centrilobular hypoxia one hour after transfusion. This alteration was not observed in rats similarly transfused with a crystalline hemoglobin solution. The absence of evidence of hypoxia was attributed to the ability of hemoglobin to transport and off-load oxygen during the immediate post-transfusion period. However, 12 hours after transfusion the livers of hemoglobin-treated animals showed centrilobular necrosis which progressed at 24 hours. It was postulated that this effect was due to the disappearance of plasma hemoglobin and to the loss of intravascular volume. To overcome this effect rats were transfused with hemoglobin solution and also received bolus infusions of hemoglobin solution at 3-hour intervals to replace plasma hemoglobin and intravascular volume lost during this time period. In the animals thus treated, normal hepatic architecture was observed 12 and 24 hours after transfusion. Similarly, all surgical control animals and those transfused with pooled rat blood had normal appearing livers. The restoration of intravascular volume with each bolus injection of hemoglobin solution was reflected in a decrease of percent packed cell volume immediately after injection. Similarly, with each bolus injection, the plasma hemoglobin concentration was returned to the immediate post-exchange level as were the oncotic pressure and the oxygen-carrying capacity. Renal function, as reflected by serum creatinine, remained stable. The blood urea nitrogen was slightly elevated at both 12 and 24 hours. The results suggest that careful maintenance of plasma hemoglobin and intravascular volume after massive transfusion with hemoglobin prevents hypoxic alterations in the liver.

Pharmacokinetic studies. In cooperation with LTC C. Peck, MC, investigations of the plasma disappearance kinetics of hemoglobin in a non-human primate have been initiated. The objectives of these studies are to characterize the routes of metabolism and excretion of hemoglobin when a non-human primate is infused with increasing doses of hemoglobin solution and to define the distribution of hemoglobin in different organs at several intervals after transfusions. In preliminary experiments, different doses of hemoglobin solution have been infused into Rhesus monkeys. Plasma and urine samples have been collected at several times after infusion, and hemoglobin concentration, osmolality, pH, and urine volume have been determined. The results indicate that at low hemoglobin concentrations (> 50 mg/dl), the plasma disappearance of hemoglobin in monkeys is of the first order with a half-time disappearance of 100 minutes.

Modification of hemoglobin. Studies reported from this and other laboratories have shown that, when hemoglobin solution is transfused in experimental animals or in humans, there is a rapid disappearance of hemoglobin from the vascular system and concomitant loss of vascular fluid volume. To overcome these difficulties it would be necessary to perform mutiple infusions of hemoglobin solution (as described above) to replace the plasma hemoglobin and the blood volume lost or to modify the hemoglobin in order to stabilize the tetrameric form and increase the vascular retention of hemoglobin. Modifications have been attempted by polymerization of hemoglobin in presence of glutaraldehyde. The results indicate that polymeric forms of hemoglobin obtained with different concentrations of glutaraldehyde have a high oxygen affinity and, at normal tissue P_{O_2} levels, practically no oxygen would be released. Experiments in which albumin, glycerol, and/or glucose were added to the hemoglobin solution before the use of glyceraldehyde showed little or no progress toward overcoming the limitations/difficulties. Studies on the encapsulation of hemoglobin molecule in artificial lipid membrane have been continued and the results show that only 10-12 percent of the total hemoglobin in solution can be encapsulated.

CONCLUSIONS

Hemoglobin, because of its ability to transport oxygen and maintain oncotic pressure, could provide the basis for a useful resuscitating solution for the severely wounded soldier. The storage stability of hemoglobin in liquid or dry form is essential for stockpiling and fulfilling the logistic requirements for supply, storage, and transport where massive fluid support is needed for the transfusion of military casualties as well as civilian casualties in accidents and mass disasters. Lyophilized hemoglobin can be maintained for several months at room temperature and requires addition of sterile water for reconstitution to a solution. A compact reverse osmosis apparatus has been evaluated for the production of purified, injectable water in field situations. This water satisfies the criteria for injectable water. The effect of rapid disappearance of plasma hemoglobin can be overcome by multiple or continuous infusion of hemoglobin solution after massive transfusion with hemoglobin solution. This restores the loss of plasma hemoglobin and of intravascular fluid volume. The hypoxic hepatic alterations with hemoglobin solution are thus prevented.

RECOMMENDATIONS

Although hemoglobin solution has the advantage of being a much better oxygen carrier than conventional plasma expanders, several issues must be resolved to clarify questions concerning transfusions of hemoglobin solution in man. Insufficient hemoglobin retention with concomitant vascular volume depletion are problems related to the dissociation of

the hemoglobin tetramer into dimer and monomer forms which disappear rapidly from the circulation. Research studies involved with modification of hemoglobin aimed at maintaining the tetrameric molecule should be intensified: such efforts may provide a stable hemoglobin compound having longer intravascular life as well as lower oxygen affinity. Quality controls in every phase of the development and evaluation of hemoglobin solution as a blood substitute should be encouraged and, at present, they are being set up. This becomes important since the hemoglobin project has become an Institute project with the participation of several investigators from different divisions within the Institute.

PUBLICATIONS

1. DEVENUTO, F., and A.I. ZEGNA. Plasma oncotic pressure during and after blood exchange with crystalline hemoglobin solution. Surg Gynecol Obstet 147:379-384, 1978

2. DEVENUTO, F. Stability of crystalline hemoglobin solution during extended storage. J Lab Clin Med 92:946-952, 1978

3. ZEGNA, A.I., K.R. BUSSE, and F. DEVENUTO. Transfusion with lyophilized reconstituted hemoglobin. Transfusion 18:633-634, 1978

4. DEVENUTO, F., A.I. ZEGNA, and K.R. BUSSE. Lyophilization of crystalline hemoglobin solution and exchange transfusions with lyophilized, reconstituted hemoglobin. Surg Gynecol Obstet 148: 69-75, 1979

5. FRIEDMAN, H.I., F. DEVENUTO, L. LOLLINI, P. MELLICK and T.F. ZUCK. Morphological effects following massive transfusions with a stroma-free hemoglobin solution. II. Kidney Lab Invest 40:655-667, 1979

6. DEVENUTO, F., H.I. FRIEDMAN, J.R. NEVILLE, and C.C. PECK. Appraisal of hemoglobin solution as a blood substitute. Surg Gynecol Obstet 149:417-436, 1979

7. DEVENUTO, F., A.I. ZEGNA, and K.R. BUSSE. Transfusions with a solution of crystalline hemoglobin supplemented with albumin. Transfusion 19:645, 1979

8. DEVENUTO, F., A.I. ZEGNA. K.R. BUSSE, and C.C. PECK. Evaluation of a reverse osmosis apparatus for field production of USP grade injectable water from sea water, pond water, and human urine. Milit Med, in press.

9. DEVENUTO, F., H.I. FRIEDMAN, and P.W. MELLICK. Massive exchange transfusions with hemoglobin solution and subsequent replacement of hemoglobin and blood volume. Morphological effects on rat liver. Surg Gynecol Obstet, in press.

STUDY NO. 6 Molecular modification of hemoglobin

PROBLEM

Two intrinsic characteristics of unmodified hemoglobin solution, namely, its increased oxygen affinity and rapid plasma clearance, impose distinct limitations on combat field use in the massively transfused soldier by requiring repeated infusions of a solution which has decreased oxygen delivery properties. The goals of molecular modification of hemoglobin have been to reduce these two problems and thus improve the solution for resuscitative purposes. Specific endpoints desired of modified hemoglobin are defined as a P_{50} between 25 and 40 torr (unmodified hemoglobin P_{50} = 13-17 torr) and a plasma retention time of 12 to 24 hours (unmodified hemoglobin plasma retention time = 2-4 hours).

RESULTS AND DISCUSSION OF RESULTS

Because of the structural and functional similarity to 2,3-diphosphoglycerate (2,3-DPG), a variety of phosphorylated mono- and disaccharides capable of selectively binding to hemoglobin by permanent covalent bond in the 2,3-DPG pocket have been studied. A chromatographic technique has been developed for detection of these modified hemoglobins. Additionally, improvement of reaction conditions to favor high yields of desired products, low yields of methemoglobin and denatured protein, and short reaction times has been studied.

Much has been learned about reaction conditions. Temperature effects are pronounced. At 4 C, the reaction rates are decreased (3.3% yield at 96 hours) so that weeks are required to attain high yields. At 40 C, although product yields increase (23.4% at 96 hours), methemoglobin (MetHb = 20-35%) formation greatly interferes with product formation. A temperature of 20 C is a practical compromise in enhancing product yields (19.2% at 96 hours) and minimizing side reactions (MetHb = 10-15%).

Yield is affected by pH varying with each sugar in an unpredictable manner. Overall the best working range is between 6.8 and 7.4. Care must be used to insure maintenance of desired pH. Increasing buffer concentration appears to increase amount of product formation.

Ionic strength of solution, although reported to enhance P_{50} of unaltered hemoglobin solution has had no apparent effect on product yield

when tested with a wide range of organic and inorganic salts. Deoxygenation of hemoglobin with hydrated argon does improve yield over oxygenated conditions by minimizing methemoglobin formation. Choice of substrates and their concentrations are critical variables. In general, rate of reaction increases as concentrations of hemoglobin and organic phosphate each increase. Methods have been developed whereby thin channel ultrafiltration can be used to concentrate hemoglobin with minimal denaturation or methemoglobin formation. The characteristics of the organic phosphate appear to be important as well. In general, highest yields of modified hemoglobin have been obtained with six and seven carbon sugars. Also, diphosphates are more reactive than monophosphates which in turn are more reactive than the unsubstituted sugars (ratio of yields under equal conditions: glucose: G6P: F16DP = 1x: 4-20x: 4x). Although no single variable to date has increased product yield above 25%, efforts are in progress to optimize all of the favorable individual variables using G6P and F16DP as model reactants to maximize yields of modified hemoglobin.

Use of techniques which minimize methemoglobin formation and protein denaturation has permitted in vitro physiologic evaluation of the reaction products. One beneficial side reaction of the organic phosphates is that in the hemoglobin reaction solution methemoglobin actually decreases from 2-10x baseline as a function of sugar type, length of reaction, and hemoglobin concentration. Oxygen affinities, as measured by P_{50}, for both the hemoglobin derivatives of G6P and F16DP have now been studied in mixture with unmodified hemoglobin. These studies reveal that both derivatives decrease oxygen affinity (increase P_{50}) as the percent of modified hemoglobin increases. Solutions containing approximately 15% of modified hemoglobin (85% unmodifed) have increased P_{50} by approximately 25% for each derivative. Work is in progress to decrease further methemoglobin formation as well as completely isolate the desired product so as to accomplish complete physiologic characterization of these modifications.

CONCLUSIONS

Several reaction variables have been found which individually increase yields of modified hemoglobin and minimize undesired side reactions. The phosphorylated sugars appear to be excellent model reactants because they do not require sodium borohydride reduction to form a covalent bond (as does pyridoxal-5-phosphate), they do promote methemoglobin reduction, and their adducts with hemoglobin do improve oxygen affinity over unmodified hemoglobin. Further studies on a variety of organic phosphates with hemoglobin in conjunction with optimized reaction conditions will provide necessary direction as to which modification is most beneficial in improving oxygen affinity and plasma clearance. Selective molecular modification of hemoglobin is promising, and the technology readily adaptable

to the enhancement of current hemoglobin solutions for use as a resuscitating solution.

RECOMMENDATIONS

Combining reaction conditions which favor maximum yields of modified hemoglobin should be optimized using G6P and F16DP as model reactants. Evaluation of in vitro hemoglobin tetramer dissociation by ultracentrifugation needs development to evaluate conveniently the stabilization of the hemoglobin tetrameric form (a measure of plasma clearance). Search for compounds capable of further improving hemoglobin resuscitative capabilities should continue until the desired level of oxygen affinity and plasma retention are achieved.

PUBLICATIONS

None

APPENDIX A

PUBLICATIONS ACCESSIONED - FISCAL YEAR 1979

INSTITUTE REPORTS

No.

58 STUCK, B.E., D.J. LUND, and E.S. BEATRICE. Repetitive pulse laser data and permissible exposure limits. October 1978

59 LEIBRECHT, B.C., A.J. LLOYD, and P.A. O'MARA. Field study of stress: Psychophysiological measures during project SUPEX. October 1978

60 SCHNAKENBERG, D.D., T.M. HILL, M.S. MORRIS, C.F. CONSOLAZIO, and J.E. CANHAM. Nutrient intakes of NAS/Alameda personnel before and after conversion to a cash a la carte food service system. November 1978

61 HILL, T.M., R.A. NELSON, C.F. CONSOLAZIO, and J.E. CANHAM. Nutrient intake of the repatriated United States Army, Navy and Marine corp prisoners-of-war of the Vietnam war. November 1978

62 KRZYWICKI, H.J., C.F. CONSOLAZIO, H.L. JOHNSON, and N.F. WITT. Effects of exercise and dietary protein levels on body composition in humans. December 1978

63 KRZYWICKI, H.J., J.A. TILLOTSON, and H.L. JOHNSON. Human body composition during riboflavin depletion of brief but sufficient uration to produce biochemical evidence of deficiency. May 1979

64 KRZYWICKI, H.J., E.M. BAKER III, J.E. CANHAM, and J. HOOD. Body composition in experimental human scurvy: A partial study. May 1979

65 KRETSCH, M.J., D.D. SCHNAKENBERG, R.D. FULTS, R.A. NELSON, Y.C. LeTELLIER, and J.E. CANHAM. Nutrient intakes and some socio-anthropometric characteristics of Twentynine Palms Marine Corps personnel before food service system modifications. June 1979

66 ESGANDARIAN, G.E., J.S. SURINCHAK, and P.A. O'MARA. Behavioral effects in rats following massive transfusion with plasma protein solution. July 1979

Publications Accessioned - Fiscal Year 1979

67 SPENCER, T.S., K.L. ZELLER, W.A. AKERS, and W.H. LANGLEY. Data storage and retrieval system for a mosquito repellent test program (Interim Report). July 1979

68 DeVENUTO, F., H.I. FRIEDMAN, J.R. NEVILLE, and C.C. PECK. Appraisal of hemoglobin solution as a blood substitute. July 1979

69 SCHUSCHEREBA, S., B. STUCK, and P. SHAWALUK. Corneal endothelium after infrared laser exposure: Evaluation of techniques to study corneal lesions. (Revised version, in process)

70 SCHUSCHEREBA, S., B. STUCK, and P. SHAWALUK. Corneal endothelium after infrared laser exposure: Results of techniques to study corneal lesions. (Paired with No. 69)

71 McGOWN, E.L., C.M. LEWIS, and P.P. WARING. Investigation of possible antithiamin properties in irradiation sterilized beef. August 1979

72 McGOWN, E.L., C.M. LEWIS, and P.P. WARING. Investigation of possible anthithiamin properties in irradiation sterilized chicken. August 1979

73 KRZYWICKI, H.J., C.F. CONSOLAZIO, H.L. JOHNSON, and N.F. WITT. Metabolic aspects of caloric restriction (500 calories) body composition changes. August 1979

74 FRUIN, J.T., H.F. ALISHOUSE, and A.L. DUNGAN. Summary of food microbiological data from the central food preparation system evaluation Fort Lee 1976 - 1978. August 1979

75 DONG, M.H., E.L. McGOWN, B.W. SCHWENNEKER, and H.E. SAUBERLICH. Vitamin assay data on food samples obtained from Naval Air Station, Alameda, CA and Marine Corps Base, Twentynine Palms, CA: Thiamin, riboflavin and vitamin B-6. August 1979

Publications Accessioned - Fiscal Year 1979

TECHNICAL NOTES

No.

TN-1 LHOTA, J., P.A. O'MARA, and J.P. HANNON. Yellow phosphorous toxicity: Pilot experiments to detect alterations in operant behavior, voluntary activity, and visual evoked potentials. June 1979

TN-2 DeVENUTO, F., and D.A. JESS. Absence of toluene in solution of hemoglobin prepared by crystallization. June 1979

TN-3 JESS, D.A., and F. DeVENUTO. Plasticizers in hemoglobin solution. June 1979

TN-4 KLAIN, G.J., and J.P. HANNON. Yellow phosphorus toxicity: In vitro lipid synthesis in the rat. June 1979

TN-5 ROBBINS, D.O., and H. ZWICK. Changes in spectral acuity following brief laser (647 nm) exposure. August 1979

TN-6 ZWICK, H., D.O. ROBBINS, and A. KNEPP. Changes in tectal spectral sensitivity and receptive field organization following coherent light exposure. In Final Draft

TN-7 ZWICK, H., E.S. BEATRICE, and T.A. GARCIA. Long-term and progressive changes in rhesus spectral sensitivity after low level coherent light (514 nm) exposure. In Final Draft

TN-8 ZWICK, H., and D.L. JENKINS. Coherency effects on retinal neural processes (ERG) of pseudemys. In Final Draft

TN-9 BLOOM, K.R., and H. Zwick. Rhesus spectral acuity for static and moving targets. August 1979

TN-10 OMARA, P.A., D.A. STAMPER, E.S. BEATRICE, D.J. LUND, R.L. JONES, R. SERENBETZ, and J.P. HANNON. Blaser: A simulator for the laboratory investigation of biomedical factors influencing laser designator operator performance. July 1979

TN-11 LHOTA, J., and J.P. HANNON. Yellow phosphorus toxicity: Body weight changes of young and mature rats during the early stages of chronic exposure. September 1979

Publications Accessioned - Fiscal Year 1979

PAPERS IN MEDICAL AND SCIENTIFIC BOOKS/JOURNALS

79-001 DEVENUTO, F., and A.I. ZEGNA. Plasma oncotic pressure during and after blood exchange with crystalline hemoglobin solution. Surg, Gynecol, Obstet 147:379-384, 1978

79-002 FRUIN, J.T., J.F. FOSTER, and J.L. FOWLER. Survey of the bacterial populations of bologna products. J Food Protect 41:692-695, 1978

79-003 TAYLOR, S.L., M. LEATHERWOOD, and E.R. LIEBER. A Survey of histamine levels in sausages. J Food Protect 41:634-637, 1978

79-004 SUDDUTH, J.H., and S.T. KELLEY. Carbamate toxicity in cats. Vet Human Toxicol 20:253-254, 1978

79-005 CHANDLER, J.S., and T.J. BUCCI. The ontogenesis of calcium-binding protein. Cell Tissue Res 191:363-365, 1978

79-006 DEVENUTO, F. Trasfusioni con emoglobina preparata mediante cristallizzazione. Trasfusione del Sangue 23:83-91, 1978

79-007 ZWICK, H., and D.O. ROBINS. Is the rhesus protanomalous? Mod Probl Ophthalmol 19:238-242, 1978

79-008 ZWICK, H., and E.S. BEATRICE. Long-term changes in spectral sensitivity after low-level laser (514nm) exposure. Mod Probl Ophthalmol 19:319-325, 1978

79-009 DEVENUTO, F. Crystalline hemoglobin solution foreseen as blood substitute. Army Res Dev Acquis 19:10, 1978

79-010 BEATRICE, E.S. Laser hazards: a vital Army research program. Army Res Dev Acquis 18: Back cover, 1977

79-011 WEISKOPF, R.B. Halothane and regulation of respiration. Anesthesiology 49:236-238, 1978

79-012 FRIEDMAN, H.I., F. DEVENUTO, L. LOLLINI, P. MELLICK, and T.F. ZUCK. Morphologic effects following massive exchange transfusions with a stroma-free hemoglobin solution I. Liver. Lab Invest 39:167-177, 1978

79-013 FRUIN, J.T. A research note. Types of Clostridium perfringens isolated from selected foods. J Food Prot 41:768-769, 1978

Publications Accessioned - Fiscal Year 1979

79-014 TAYLOR, S.L., E.R. LIEBER, and M. LEATHERWOOD. A survey of histamine levels in commercially processed scombroid fish products. J Food Qual 1:393-397, 1977-78

79-015 SHERR, H.P., F.B. STIFEL, and R.H. HERMAN. Effect of cholera toxin on rabbit jejunal carbohydrate-metabolizing enzymes. Gastroenterology 75:711-716, 1978

79-016 GUTHERTZ, L.S., and J.T. FRUIN. Yersinia enterocolitica: a foodborne pathogen. Army Veterinary Corps Information. Washington, DC: Department of the Army, Office of the Surgeon General, November-December 1978

79-017 MORRISSEY, R.L., D.T. ZOLOCK, T.J. BUCCI, and D.D. BIKLE. Immunoperoxidase localization of vitamin D dependent calcium binding protein. J Histochem 26:628-634, 1978

79-018 AKERS, W.A., and D.N. NAVERSEN. Diagnosis of chronic urticaria. Int J Dermatol 17:616-627, 1978

79-019 WIRTZ, R.A., S.L. TAYLOR, and H.G. SEMEY. Concentrations of substituted ρ-benzoquinones and 1-pentadecene in the flour beetles Tribolium confusum J. Du Val and Tribolium castaneum (Herbst). Comp Biochem Physiol [B] 61:25-28, 1978

79-020 CHILDS, G.E., K.A. FOSTER, and M.J. McROBERTS. Insect cell culture media for cultivation of New World Leishmania. Int J Parasitol 8: 255-258, 1978

79-021 CABAUD, H.E., and W.G. RODKEY. Use of a lightweight, durable, thermoplastic cast material. Am J Sports Med 6: 409, 1978

79-022 WIRTZ, R.A., S.L. TAYLOR, and H.G. SEMEY. Concentrations of substituted ρ-benzoquiones and 1-pentadecene in the flour beetles Tribolium madens (Charp.) and Tribolium brevicornis (Lec.) (Coleoptera, Tenebrionidae). Comp Biochem Physiol [C] 61: 287-290, 1978

79-023 LIEBER, E.R., and S.L. TAYLOR. Comparison of thin-layer chromatographic detection methods for histamine from food extracts. J Chromatogr 160: 227-237, 1978

79-024 McGOWN, E.L., C.M. LEWIS, M.H. DONG, and H.E. SAUBERLICH. Results with commercial radioassay kits compared with microbiological assay of folate in serum and whole-blood. Clin Chem 24: 2186-2191, 1978

Publications Accessioned - Fiscal Year 1979

79-025 KERBS, S., and A.M. ALLEN. Effect of occlusion of Trichophyton mentagrophytes infections in guinea pigs. J Invest Dermatol 71: 301-304, 1978

79-026 FOSTER, J.F., L.S. GUTHERTZ, R.C. HUNDERFUND, and J.L. FOWLER. Comparison of bacterial species isolated from ground beef, textured soy protein and ground beef extended with textured soy protein. J Food Prot 41: 961-964, 1978

79-027 DeVENUTO, F., A.I. ZEGNA, and K.R. BUSSE. Lyophilization of crystalline hemoglobin solution and exchange transfusions with lyophilized, reconstituted hemoglobin. Surg Gynecol Obstet 148: 69-75, 1979

79-028 ZWICK, H. Low level laser light effects. Proc Soc Photo-Opt Instrum Eng 162: 112-118, 1978

79-029 STUCK, B.E., and D.J. LUND. Extrapolation of pulsed light data in scanned displays. Proc Soc Photo-Opt Instrum Eng 162: 107-111, 1978

79-030 BEATRICE, E.S., and S. VELEZ. Laser safety bioeffects research: Histopathology of retinal effects. Proc Soc Photo-Opt Instrum Eng 162: 103-106, 1978

79-031 RUTLEDGE, L.C., R.K. SOFIELD, and M.A. MOUSSA. A bibliography of diethyl toluamide. Bull Entomol Soc Am 24: 431-439, 1978

79-032 DeVENUTO, F. Stability of hemoglobin solution during extended storage. J Lab Clin Med 92: 946-952, 1978

79-033 TAYLOR, S.L., L.S. GUTHERTZ, M. LEATHERWOOD, F. TILLMAN, and E.R. LIEBER. Histamine production by food-borne bacterial species. J Food Safety 1: 173-187, 1978

79-034 TILLOTSON, J.A. Ascorbate metabolism in the trained monkey. Int J Vitam Nutr Res 48: 374-381, 1978

79-035 LUND, D.J., and E.S. BEATRICE. Ocular hazard of short pulse argon laser irradiation. Health Phys 36: 7-11, 1979

79-036 ZUCK, T.F., F. DeVENUTO, J.R. NEVILLE, and H.I. FRIEDMAN. Oncotic and oxygen transport effects of hemoglobin solutions. In: Blood Substitutes and Plasma Expanders, edited by G.A. Jamieson. New York: Alan R. Liss, 1978

Publications Accessioned - Fiscal Year 1979

79-037 HERMAN, R.H., and L. HAGLER. Food intolerance in humans. West J Med 130: 95-116, 1979

79-038 CABAUD, H.E., W.G. RODKEY, and J.A. FEAGIN. Experimental studies of acute anterior cruciate ligament injury and repair. Am J Sports Med 7: 18-22, 1979

79-039 MOORE, G.L., M.E. LEDFORD, and D.E. BROOKS. Plasma adenine and cellular ATP in red cell concentrates collected and stored in modified CPD at 4 C. Transfusion 19: 68-71, 1979

79-040 HERMAN, R.H., and L. HAGLER. Clinical nutrition is a medical specialty. West J Med 130: 93-94, 1979

79-041 RUTLEDGE, L.C., M.A. LAWSON, L.L. YOUNG, and M.A. MOUSSA. Rearing of Diamanus montanus (Siphonaptera: Ceratophyllidae) and Hoplopsyllus anomalus (Siphonaptera: Pulicidae). J Med Entomol 15: 304, 1979

79-042 KEITH, W.A., R.J. SMILJANIC, W.A. AKERS, and L.W. KEITH. Uneven distribution of aerobic mesophilic bacteria on human skin. Appl Environ Microbiol 37: 345-347, 1979

79-043 TAYLOR, S.L., L.S. GUTHERTZ, M. LEATHERWOOD, and E.R. LIEBER. Histamine production by Klebsiella pneumoniae and an incident of scombroid fish poisoning. Appl Environ Microbiol 37: 274-278, 1979

79-044 FRUIN, J.T., L.S. GUTHERTZ, and W.S. CLARK. The precision, consistency and speed of automatic colony counting devices in the enumeration of bacterial colonies on agar plates. (Abstract No. P9) In: Abstracts of the Annual Meeting of the American Society for Microbiology (Los Angeles, CA, 4-8 May 1979

79-045 FRUIN, J.T., L.S. GUTHERTZ, and H.E. SAUBERLICH. Survival of pathogenic bacteria in foods prepared in a microwave oven. (Abstract No. P34) In: Abstracts of the Annual Meeting of the American Society for Microbiology (Los Angeles, CA, 4-8 May 1979

79-046 FRUIN, J.T. Food microbiological criteria. In: Memorandum for: Each Army Veterinary Corps Officer, Letter of March 1979, Subject: Army Veterinary Corps Information (2-79) 4 pp

79-047 BIKLE, D.D., L. HAGLER, L.O. LOLLINI, S.F. HULL, and R.H. HERMAN. Work-induced muscle hypertrophy in vitamin D-deficient rats. Am J Clin Nutr 32: 515-516, 1979

Publications Accessioned - Fiscal Year 1979

79-048 SHROYER, E.L., S.T. KELLEY, P.C. TAYLOR, P. VANDERLOO, and T.L. LESTER. Three serologic types of adenovirus infections of owl monkeys. Am J Vet Res 40: 532-536, 1979

79-049 BIBEL, D.J., and R.J. SMILJANIC. Interactions of Trichophyton mentagrophytes and micrococci on skin culture. J Invest Dermatol 72: 133-137, 1979

79-050 OMAYE, S.T., and J.D. TURNBULL. Heme oxygenase activity, drug metabolism, and ascorbic acid distribution in the livers of ascorbic acid-deficient guinea pigs. Biochem Pharmacol 28: 1415-1419, 1979

79-051 GUTHERTZ, L.S., and J.T. FRUIN. Colony count accuracy using selective media: Analysts versus automatic colony counters. J Food Prot 42: 420-423, 1979

79-052 WILSON, H.R., B.S. DIECKMANN, and G.E. CHILDS. Leishmania braziliensis and Leishmania mexicana: Experimental cutaneous infections in golden hamsters. Exp Parasitol 47: 270-283, 1979

79-053 TURNBULL, J.D., H.E. SAUBERLICH, and S.T. OMAYE. Effects of ascorbic acid deficiency and of erythorbic acid on blood components in the Cynomolgus monkey. Int J Vitam Nutr Res 49: 92-102, 1979

79-054 TAYLOR, S.L., and E.R. LIEBER. Subcellular distribution and properties of intestinal histamine-metabolizing enzymes from rats, guinea pigs and rhesus monkeys. Comp Biochem Physiol 63C: 21-26, 1979

79-055 PECK, C.C., D.G. ODOM, H.I. FRIEDMAN, P.W. ALBRO, J.R. HASS, J.T. BRADY, and D.A. JESS. Di-2-ethylhexyl phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) accumulation in whole blood and red cell concentrates. Transfusion 19: 137-146, 1979

79-056 HILL, J.A., P.B. ROBINSON, D.L. McVEY, W.A. AKERS, and W.G. REIFENRATH. Evaluation of mosquito repellents on the hairless dog. Mosquito News 39: 307-310, 1979

79-057 BIKLE, D.D., R.L. MORRISSEY, D.T. ZOLOCK, and R.H. HERMAN. Stimulation of chick gut alkaline phosphatase activity by actinomycin D and 1,25-dihydroxyvitamin D_3: Evidence for independent mechanisms. J Lab Clin Med 94: 88-94, 1979

Publications Accessioned - Fiscal Year 1979

79-058 TAYLOR, S.L., and E.R. LIEBER. In vitro inhibition of rat intestinal histamine-metabolizing enzymes. Food Cosmet Toxicol 17: 237-240, 1979

79-059 GREEN, M.D., and S.T. OMAYE. Effect of thiamin status on drug metabolism in mice. Proc West Pharmacol Soc 22: 129-132, 1979

79-060 GREEN, M.D., W. BELL, C. KRAUT, and S. OMAYE. Failure of ascorbic acid to influence brain catecholamines in the guinea pig. Experientia 35: 515, 1979

79-061 LEDFORD, M.E., and G.L. MOORE. The formulation of citrate-phosphate-dextrose anticoagulant. Letter to the Editor. Transfusion 19: 350, 1979

79-062 FRIEDMAN, H., F. DeVENUTO, L. LOLLINI, P. MELLICK, and T.F. ZUCK. Morphologic effects following massive exchange transfusions with a stroma-free hemoglobin solution. II. Kidney. Lab Invest 40: 655-667, 1979

79-063 DeVENUTO, F., H.I. FREIDMAN, J.R. NEVILLE, and C.C. PECK. Appraisal of hemoglobin solution as a blood substitute. Surg Gynecol Obstet 149: 417-436, 1979

79-064 HERMAN, R.H. The interaction between the gastrointestinal tract and nutrient intake. Chapter 4. In: Human Nutrition; A Comprehensive Treatise, Vol 4, edited by R.B. Aifin-Slater and D. Kritchevsky. New York: Plenum, 1979

79-065 HAGLER, L., R.I. COPPES, and R.H. HERMAN. Metmyoglobin reductase. Identification and purification of the reduced nicotinamide adenine dinucleotide-dependent enzyme from bovine heart which reduces myoglobin. J Biol Chem 254: 6505-6514, 1979

79-066 LUZZIO, A.J., M.J. McROBERTS, and N.H. EULISS. Quantitative estimation of leishmanial antibody titers by enzyme-linked immunosorbent assay. J Infect Dis 140: 370-377, 1979

79-067 CHILDS, G.E., M.J. McROBERTS, and M.A. MOUSSA. Systems for the in vitro large-scale propagation of New World Leishmania. Ann Trop Med Parasitol 73: 395-396, 1979

79-068 SPENCER, T.S., J.A. HILL, R.J. FELDMANN, and H.I. MAIBACH. Evaporation of diethyltoluamide from human skin in vivo and in vitro. J Invest Dermatol 72: 317-319, 1979

Publications Accessioned - Fiscal Year 1979

79-069 SKINNER, W.A., H.T. CRAWFORD, L.C. RUTLEDGE, and M.A. MOUSSA. Topical mosquito repellents. XI. Carbamates derived from N, N'-distributed diamines. J Pharm Sci 68: 390-391, 1979

79-070 SKINNER, W.A., H.T. CRAWFORD, L.C. RUTLEDGE, and M.A. MOUSSA. Topical mosquito repellents. XII. N-substituted ureas and cyclic ureas. J Pharm Sci 68: 391-392, 1979

79-071 MOORES, W.Y., R.B. WEISKOPF, W.P. DEMBITSKY, and J.R. UTLEY. Comparative effects of halothane and morphine anesthesia on myocardial function and metabolism during cyanosis in swine. Surg Forum 30: 221-223, 1979

79-072 WATSON, L.C., and R. GOMEZ. Effect of hydrocortisone on gastric acid secretion and plasma amino acids in dogs. Surg Forum 30: 343-347, 1979

79-073 KERBS, S., R. HUTTON, M. LANCASTER, and K. JESRANI. Effects of deferoxamide methanesulfonate on Trichophyton mentagrophytes. Sabourandia 17: 241-250, 1979

79-074 CANHAM, J.E. The biomedical and behavioral basis of clinical nutrition: A projection for the 1980s. In: NIH Publication *No. 80-1970. Report of a* National Conference Sponsored by the Nutrition Coordinating Committee, National Institutes of Health, 19-20 June 1978, edited by A. P. Simopoulos. U.S. Department of Health, Education, and Welfare (Public Health Service, National Institutes of Health) November 1979. Washington, DC: U.S. Government Printing Office, 1979, pp 65-69

79-075 MORRIS, R.E., E. ROSENTAL, R.H. HERMAN, and M.M. THALER. Mechanism of fasting hyperbilirubinemia in Gilbert's Syndrome: Inhibition of lipolysis by dietary fat. (Abstract) Gastroenterology 75: 978, 1978

79-076 ROSENTAL, E., R.E. MORRIS, R.H. HERMAN, and M.M. THALER. Opposing effects of nicotinic acid on bilirubin metabolism. (Abstract) Gastroenterology 75: 984, 1978

79-077 SAUBERLICH, H.E. Laboratory indices of nutritional status. Nutrition & the M.D. V(5): 1-2, 1979

79-078 BRUBACHER, G.B., H.F. DeLUCA, D.R. FRASER, H.E. SAUBERLICH, and V.B. SPIRICHEV. Research priorities in the field of vitamin deficiency in man. Am J Clin Nutr 32: 1982-1983, 1979

Publications Accessioned - Fiscal Year 1979

79-079 SAUBERICH, H.E., Y.F. HERMAN, C.O. STEVENS, and R.H. HERMAN. Thiamin requirement of the adult human. Am J Clin Nutr 32: 2237-2248, 1979

79-080 BRINK, E.W., W.D.A. PERERA, S.P. BROSKE, R.A. CASH, J.L. SMITH, H.E. SAUBERLICH, and M.M. BASHOR. Vitamin A status of children in Sri Lanka. Am J Clin Nutr 32: 84-91, 1979

79-081 KNUDSEN, J.J., E.W. ASKEW, J.F. LOWDER, JR., S.S. KULINSKI, and G.J. KLAIN. Dietary triundecanoin as a gluconeogenic energy source of exercising rats. (Abstract No.2044) Fed Proc 38: 614, 1979

79-082 MILNE, D.B., and S.T. OMAYE. Effect of ascorbic acid on copper and iron in the guinea pig and the monkey. (Abstract No. 2044) Fed Proc 38: 619, 1979

79-083 OMAYE, S.T., and J.D. TURNBULL. Influence of ascorbic acid (AA) on hepatic microsomal heme oxygenase (MHO) and cytochrome P-450 in the guinea pig (GP). (Abstract No. 185) Pharmacologist 20: 181, 1978

79-084 OMAYE, S.T., J.D. TURNBULL, and H.E. SAUBERLICH. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Chapter 1. In: Methods in Enzymology, Volume 62. Academic Press, Inc., 1979. pp 3-11

79-085 OMAYE, S.T., J.D. TURNBULL, and D.B. MILNE. Influence of ascorbic acid on hepatic cytochrome P-450 heme metabolism in the guinea pig. (Abstract No. 04-2-S69) In: XI. International Congress of Biochemistry (Toronto, Canada) 1979. p 263

79-086 CROSS, C.E., S.T. OMAYE, D.C. RIFAS, G.K. HASEGAWA, and K.A. REDDY. Biochemical effects of intratracheal instillation of cadmium chloride on the rat lung. Biochemical Pharmacol 28: 381-388, 1979

79-087 ASKEW, E.W., G.J. KLAIN, J.F. LOWDER, JR., and W.R. WISE. Influence of exercise on amino acid mobilization and oxidation. (Abstract No. 2) Med Sci Sports 11: 106, 1979

79-088 SCOTT, R.L., J.E. ALBINA, and M.D. CALDWELL. Hypocaloric feeding: Effect of carbohydrate and amino acid exchange. (Abstract) J Parent Enter Nutr 3: 22, 1979

Publications Accessioned - Fiscal Year 1979

79-089 O'CONNOR, J.A., J.L. KROPP, S.L. HULL, and M.D. CALDWELL. The effect of somatomedin on glucose stimulated gluconeogenesis. (Abstract) J Parent Enter Nutr 3: 22, 1979

79-090 SHEARER, J.D., G. CARPENTER, S. PETERSON, G. SHELDON, and M.D. CALDWELL. Effect of intravenous nutrient supply on plasma fatty acid composition. (Abstract) J Parent Enter Nutr 3: 22, 1979

79-091 CALDWELL, M.D., H.I. FRIENDMAN, L. LOLLINI, J. SHEARER, and T.J. NEMETH. Effect of essential fatty acid deficiency (EFAD) on the induction of hepatic microsomal membrane proliferation by phenobarbital. (Abstract) J Parent Enter Nutr 3: 22, 1979

79-092 RODKEY, W.G., H.E. CABAUD, and H.R. McCARROLL. Neurorrhaphy after loss of a nerve segment: Comparison of epineurial suture under tension versus multiple nerve grafts. (Abstract) J Hand Surg 4: 283, 1979

79-093 CABAUD, H.E., W.G. RODKEY, and J.A. FEAGIN. Experimental studies of acute anterior cruciate ligament injury and repair. (Abstract) Orthop Trans 3: 98, 1979

79-094 HEYDORN, W.H., J.W. GEASLING, W.Y. MOORES, L.O. LOLLINI, and A.C. GOMEZ. Changes in the manufacture of expanded microporous polytetrafluoroethylene: Effects on patency and histological behavior when used to replace the superior vena cava. Ann Thorac Surg 27: 173-177, 1979

79-095 HEYDORN, W.H., W.Y. MOORES, J. MACK, and W.P. DEMBITSKY. The importance of hyperkalemia in a cold perfusion solution: A correlative study examining myocardial function, metabolism, tissue gases and substrates. Ann Thorac Surg 28: 281-289, 1979

79-096 KIESLING, V.J., H.I. FRIEDMAN, J.W. McANINCH, D.A. NACHTSHEIM, and T.J. NEMETH. Ultrastructural changes in prostate adenocarcinoma following external beam radiation therapy. J Urol 122: 633-636, 1979

79-097 HANNON, J.P. Comparative altitude adaptability of young men and women. In: Proceedings International Symposium, Environmental Stress, edited by L.J. Folinsbee, et al.: New York, Academic Press, 1978. pp 335-350

79-098 WILLIAMS, J.E., M.A. MOUSSA, and D.C. CAVANAUGH. Experimental plague in the California ground squirrel. J Infect Dis 140: 618-621, 1979

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